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Enzymatic assembly of carbon–carbon bonds via iron–catalysed sp^3 C–H functionalization

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Supplementary Information

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This PDF file includes:

I. General methods	2–6
II. Supplementary Figs. 1–11 and Supplementary Tables 1–5	7–20
III. Screening of enzymes for C–H alkylation activity	21–24
IV. Directed evolution of C–H alkylation enzymes	25–30
V. Nucleotide and amino acid sequences	31–35
VI. Substrate synthesis and characterization	36–43
VII. Synthesis and characterization of reference compounds	44–53
VIII. Small scale enzymatic reactions and product calibration curves	54–65
IX. Enzymatic C–H alkylation reactions on preparative scale	66–76
X. Syntheses of (+)-lyngbic acid and (+)-cuspareine	77–81
XI. Determination of enantioselectivity	82–106
XII. NMR Spectra	107–156
XIII. Supplemental References	157–160

I. General methods

(A) General. Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers (Sigma-Aldrich, VWR, Alfa Aesar, Combi-Blocks) and used without further purification. Silica gel chromatography was carried out using AMD Silica Gel 60, 230-400 mesh. ^1H and ^{13}C NMR spectra were recorded on a Varian Inova 300 MHz, Varian Inova 500 MHz, or Bruker Prodigy 400 MHz instrument in CDCl_3 and are referenced to residual protio solvent signals. Data for ^1H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets), coupling constant (Hz), integration. Sonication was performed using a Qsonica Q500 sonicator. High-resolution mass spectra were obtained at the California Institute of Technology Mass Spectral Facility. Synthetic reactions were monitored by thin layer chromatography (TLC, Merck 60 gel plates) using a UV-lamp or an appropriate TLC stain for visualization.

E. coli cells were grown using Luria-Bertani medium (LB) or Hyperbroth (AthenaES) (HB) with 0.1 mg/mL ampicillin (LB_{amp} or HB_{amp}). Primer sequences are available upon request. T5 exonuclease, Phusion polymerase, and Taq ligase were purchased from New England Biolabs (NEB, Ipswich, MA). M9-N minimal medium (abbreviated as M9-N buffer; pH 7.4) was used as a buffering system for whole cells and lysates, unless otherwise specified. M9-N buffer was used without a nitrogen source; it contains 47.7 mM Na_2HPO_4 , 22.0 mM KH_2PO_4 , 8.6 mM NaCl, 2.0 mM MgSO_4 , and 0.1 mM CaCl_2 .

(B) Chromatography. Analytical reversed-phase high-performance liquid chromatography (HPLC) was carried out using an Agilent 1200 series instrument and a Kromasil 100 C18 column (4.6×50 mm, 5 μm) or an Eclipse XDB C18 column (4.6×150 mm, 3 μm) with water and acetonitrile as the mobile phase. Analytical chiral HPLC was conducted using either an Agilent 1200 series instrument with *n*-hexane and isopropanol as the mobile phase or JACSO 2000 series supercritical fluid chromatography (SFC) system with supercritical CO_2 and isopropanol as the mobile phase. Enantiomers were separated using one of the following chiral columns: Chiralpak AD-H, Chiralpak IC (4.6 mm \times 25 cm), Chiralcel OB-H (4.6 mm \times 25 cm), Chiralcel OD-H (4.6 mm \times 25 cm), Chiralcel OJ-H (4.6 mm \times 25 cm). Gas chromatography (GC) analysis was carried out using an Agilent 7820A or Shimadzu GC-17A GC system, both equipped with an FID detector and with a J&W HP-5 column (30 m \times 0.32 mm, 0.25 μm film). Chiral GC was conducted using either an Agilent 7820A instrument (FID) and an Agilent CycloSil-B column (30 m \times 0.32 mm, 0.25 μm film) or an Agilent 6850 GC (FID) with a Chiraldex G-TA column (30 m \times 0.25 mm, 0.12 μm film). Gas chromatography-mass spectrometry (GC-MS) analyses were carried out using a Shimadzu GCMS-QP2010SE system and J&W HP-5ms column (30 m \times 0.25 mm, 0.25 μm film).

(C) Cloning and site-saturation mutagenesis. pET22b(+) was used as a cloning and expression vector for all enzymes described in this study. All enzymes described in this study were expressed with a C-terminal 6xHis-tag. Site-saturation mutagenesis was performed using the “22c-trick” method¹. The PCR products were digested with *DpnI*, gel purified, and ligated using Gibson MixTM (ref. 2). The ligation mixture was used to directly transform electrocompetent *E. coli* strain *E. coli* BL21(DE3) cells (Lucigen).

(D) Expression of P450 and P411 variants in 96-well plates. Single colonies from LB_{amp} agar plates were picked using sterile toothpicks and cultured in deep-well 96-well plates containing

LB_{amp} (300 μ L/well) at 37 °C, 220 rpm shaking, and 80% relative humidity overnight. After, HB_{amp} (1000 μ L/well) in a deep-well 96-well plate was inoculated with an aliquot (50 μ L/ well) of these overnight cultures and allowed to shake for 3 hours at 37 °C, 220 rpm, and 80% relative humidity. The plates were cooled on ice for 30 minutes and the cultures were induced with 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at 20 °C, 150 rpm for 16–20 hours.

(E) Reaction screening in 96-well plate format. *E. coli* (*E. coli* BL21(DE3)) cells in deep-well 96-well plates were pelleted ($3,000 \times g$, 5 min, RT) and resuspended in M9-N buffer (20 μ L/well) by gentle vortexing. A GOX oxygen depletion system was added (20 μ L/well of a stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate buffer, pH 8.0) and the 96-well plate was transferred into an anaerobic chamber. In the anaerobic chamber, argon-sparged reaction buffer (50 mM glucose in M9-N or 33 mM glucose in M9-N, 300 μ L/well) was added, followed by alkane substrate (10 μ L/well, 400 mM in EtOH) and ethyl diazoacetate (10 μ L/well, 400 mM in EtOH). In some cases, the substrates and reaction buffer were mixed together prior to addition to the plate. The plate was sealed with an aluminum foil and shaken at room temperature and 500 rpm in the anaerobic chamber. After 5–20 hours, the seal was removed and the reactions were worked up following the appropriate method below.

Product formation screening using GC and GC-MS. After 5–20 hours, a solution of 0.4 mM 1,3,5-trimethoxybenzene (internal standard) in a mixed solvent system (cyclohexane/ ethyl acetate = 1:1, 510 μ L) was added. The plate was tightly sealed with a reusable silicone mat, vortexed (15 s \times 3) and centrifuged ($3,000 \times g$, 5 min) to completely separate the organic and aqueous layers. The organic layers (180 μ L/well) were transferred to 300 μ L vial inserts, which were then placed in 2 mL vials and analyzed by GC.

Product formation screening using HPLC. After 5–20 hours, the reaction mixtures, or an aliquot thereof (150 μ L/well), were quenched by the addition of an equal or greater volume of acetonitrile (400 μ L/well or 150–200 μ L/well). This step is kept consistent within each round of directed evolution. The plate containing the resulting mixture was tightly sealed with a reusable silicone mat, vortexed (15 s \times 3) and centrifuged ($3,000 \times g$, 5 min) to pellet the cells. The supernatant was filtered through an AcroPrep 96-well filter plate (0.2 μ m) into a shallow-well plate and analyzed by reverse-phase HPLC.

Enantioselectivity screening. After 5–24 hours, mixed solvent (cyclohexane/ ethyl acetate = 1:1, 250–500 μ L/ well) was added to the reaction mixtures or aliquots thereof (250 μ L). The plate containing the resulting mixture was tightly sealed with a reusable silicone mat, vortexed (15 s \times 3) and centrifuged ($3,000 \times g$, 5 min) to completely separate the organic and aqueous layers. When smaller volumes of mixed solvent were used for the extraction (< 400 μ L), the extraction mixture was transferred to a 1.6 mL Eppendorf tube, vortexed (15 s \times 3), and centrifuged ($20,000 \times g$, 1 min). The organic layers (180 μ L/well) were transferred to 300 μ L vial inserts, which were then placed in 2 mL vials and analyzed by chiral HPLC (IC column, 2% *i*-PrOH in *n*-hexane).

(F) Expression of P411 variants. *E. coli* (*E. coli* BL21(DE3)) cells carrying plasmid encoding the appropriate P411 variant were grown overnight in 5 mL LB_{amp}. Preculture (2 mL) was used to inoculate 48 mL of HB_{amp} in a 125 mL Erlenmeyer flask; this culture was incubated at 37 °C, 230 rpm for 2.5 hours. The culture was then cooled on ice (20–30 min) and induced with 0.5 mM IPTG and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at 20 °C, 130 rpm, for 16–18 hours. Following, *E. coli* cells were pelleted by centrifugation (2,600 × g, 10 min, 4 °C or 3,000 × g, 5 min, 4 °C). Media was removed and the resulting cell pellet was resuspended in M9-N buffer to OD₆₀₀ = 60. An aliquot of this cell suspension (3 mL) was taken to determine P411 concentration using the hemochrome assay after lysis by sonication. When applicable, remaining cell suspension was further diluted with M9-N buffer to the OD₆₀₀ used for the biotransformation (described in **(H)**) and the concentration of P411 protein in the biotransformation was calculated accordingly.

(G) Hemochrome assay for the determination of heme protein concentration. *E. coli* cells expressing heme protein and resuspended in M9-N buffer were lysed by sonication using a Qsonica Q500 sonicator equipped with a microtip (2 mins, 1 second on, 1 second off, 25% amplitude); samples were kept on wet ice for this process. The resulting lysed solution was centrifuged (20,000 × g, 10 min, 4 °C) to remove cell debris. The supernatant (clarified lysate) was separated from the pellet and kept on ice until use. In a falcon tube, a solution of 0.2 M NaOH, 40% (v/v) pyridine, 0.5 mM K₃Fe(CN)₆ was prepared (pyridine-NaOH-K₃Fe(CN)₆ solution). Separately, a solution of 0.5 M Na₂S₂O₄ (sodium dithionite) was prepared in 0.1 M NaOH. To an Eppendorf tube containing 500 µL of clarified lysate in M9-N buffer was added 500 µL of the pyridine-NaOH-K₃Fe(CN)₆ solution, mixed, and transferred to a cuvette; the UV-Vis spectrum of the oxidized Fe^{III} state was recorded immediately. To the cuvette was then added 10 µL of the sodium dithionite solution. The cuvette was sealed with parafilm and the UV-Vis spectrum of the reduced Fe^{II} state was recorded immediately. A cuvette containing 500 µL of M9-N, 100 µL 1 M NaOH, 200 µL pyridine, and 200 µL water (complete mixture without protein and K₃Fe(CN)₆) was used as a reference for all absorbance measurements. Concentrations of cytochromes P450, cytochromes P411, and globins were determined using a published extinction coefficient for heme *b*, $\epsilon_{556(\text{reduced})-540(\text{oxidized})} = 23.98 \text{ mM}^{-1}\text{cm}^{-1}$ (ref. 3). Cytochrome *c* concentration was measured using a modified procedure, reported previously⁴.

(H) Biotransformations using whole *E. coli* cells. Suspensions of *E. coli* (*E. coli* BL21(DE3)) cells expressing the appropriate heme protein variant in M9-N buffer (typically OD₆₀₀ = 30) were degassed by bubbling with argon in sealed vials for at least 40 minutes; the cells were kept on ice during this time. Separately, a solution of *D*-glucose (250 mM in M9-N) was degassed by sparging with argon for at least 30 minutes. All solutions were then transferred into an anaerobic chamber for reaction set up. To a 2 mL vial were added a GOX oxygen depletion solution (20 µL of stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate buffer, pH 8.0), *D*-glucose (40 µL of 250 mM stock solution in M9-N buffer), degassed suspension of *E. coli* expressing P411 (typically OD₆₀₀ = 30, 320 µL), alkane substrate (10 µL of 400 mM stock solution in EtOH), and diazo compound (10 µL of 400 mM stock solution in EtOH) in the listed order. Final reaction volume was 400 µL; final concentrations were 10 mM alkane substrate, 10 mM diazo compound, and 25 mM *D*-glucose. **Note:** reaction performed with *E. coli* cells resuspended to OD₆₀₀ = 30 indicates that 320 µL of OD₆₀₀ = 30 cells were added, and likewise for other reaction OD₆₀₀ descriptions. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature and 500 rpm for 18 hours. A modified procedure was used for reactions conducted at 4 °C. Reactions were set up in the same manner, except kept on

ice. Reactions were shaken in a cold room (4 °C) and 500 rpm for 18 hours. The reactions were worked up and analyzed by HPLC or GC as appropriate; the reaction workup procedures are outlined in detail in **Section VIII**. Preparative scale enzymatic reactions were performed using a different procedure which is described in detail in **Section IX**. The expression of heme protein was measured using the hemochrome assay (**Section I (G)**), and the concentration of heme protein in the biotransformation was calculated accordingly.

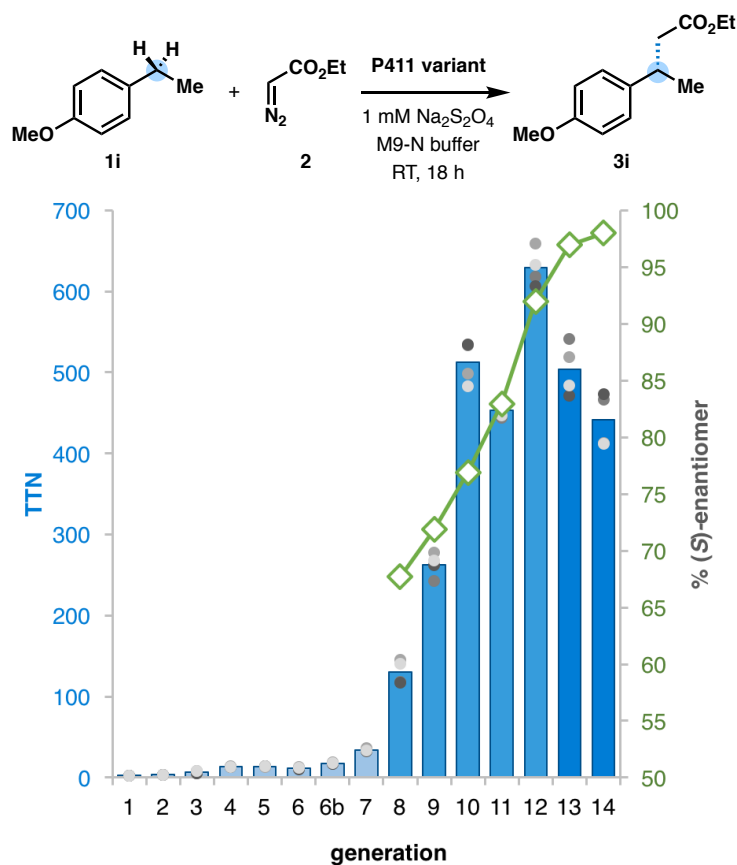
(I) Enzymatic reactions using clarified *E. coli* lysate. Lysates for biocatalytic reactions were prepared as follows: *E. coli* (*E. coli* BL21(DE3)) cells expressing the appropriate heme protein variant were resuspended in M9-N buffer and adjusted to OD₆₀₀ = 60. The cell suspension, in 3 mL portions, was lysed by sonication using a Qsonica Q500 sonicator equipped with a microtip (2 mins, 1 second on, 1 second off, 25% amplitude); samples were kept on wet ice for this process. The resulting lysed solution was centrifuged (20,000 × g, 10 min, 4 °C) to remove cell debris. Protein concentration of the supernatant (clarified lysate) was determined using the hemochrome assay (see **Section I (G)**); the protein concentration in lysate was adjusted to the desired amount by addition of M9-N buffer. Lysate was placed in a sealed vial and the headspace of the vial was purged with a stream of argon for at least 40 minutes. The lysate was kept on ice during all parts of this procedure. Separately, *D*-glucose solution (500 mM in M9-N buffer) and Na₂S₂O₄ (20 mM in M9-N) were degassed by bubbling the solutions with argon for at least 40 minutes. All solutions were then transferred into an anaerobic chamber for reaction set up. To a 2 mL vial were added a GOX oxygen depletion solution (20 µL of stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate buffer, pH 8.0), *D*-glucose (20 µL of 500 mM stock solution in M9-N buffer), lysate (320 µL), Na₂S₂O₄ (20 µL of 20 mM solution in M9-N), alkane substrate (10 µL of 400 mM stock solution in EtOH), and ethyl diazoacetate (10 µL of 400 mM stock solution in EtOH) in the listed order. Final reaction volume was 400 µL; final concentrations were typically 2.0 µM heme protein, 1 mM Na₂S₂O₄, 10 mM alkane substrate, 10 mM ethyl diazoacetate, and 25 mM *D*-glucose. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature and 500 rpm for 18 hours. Reactions were analyzed following the same methods as described for biotransformations using whole *E. coli* cells (see **Section VIII**).

(J) Protein purification. *E. coli* (*E. coli* BL21(DE3)) cells carrying plasmid encoding a P411 variant were grown overnight in 19 mL LB_{amp} (37 °C, 250 rpm). HB_{amp} (450 mL) in a 1 liter flask was inoculated with 19 mL of the preculture and shaken for 2.5 hours at 37 °C and 220 rpm. Cultures were cooled on ice (30 min) and induced with 0.5 mM IPTG and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at 22 °C, 130 rpm, for 16–20 hours. Cultures were then centrifuged (5,000 × g, 10 min, 4 °C) and the cell pellets frozen at -20 °C. For purification, frozen cells from two such cultures were resuspended in His-trap buffer A (25 mM tris, 100 mM NaCl, 20 mM imidazole, pH 7.5, 4 mL/g of cell wet weight), loaded with hemin (1 mg/g wet cell weight) and powdered DNaseI, and lysed by sonication. To pellet cell debris, lysates were centrifuged (20,000 × g, 20 min, 4 °C). The protein containing a C-terminal 6xHis-tag was purified with a nickel NTA column (1 mL HisTrap HP, GE Healthcare, Piscataway, NJ) using an AKTA purified FPLC system (GE healthcare). Proteins were eluted on a linear gradient from His-trap buffer A to His-trap buffer B (25 mM tris, 300 mM imidazole, 100 mM NaCl, pH 7.5) over 10 column volumes. Fractions containing the desired heme protein were combined, concentrated, and subjected to three exchanges of phosphate buffer (0.1 M potassium phosphate, pH 8.0) using centrifugal filters (10 kDa molecular weight cut-off, Amicon Ultra, Merck Millipore) to remove excess salt and imidazole. Concentrated proteins were aliquoted,

flash-frozen on powdered dry ice, and stored at -80 °C.

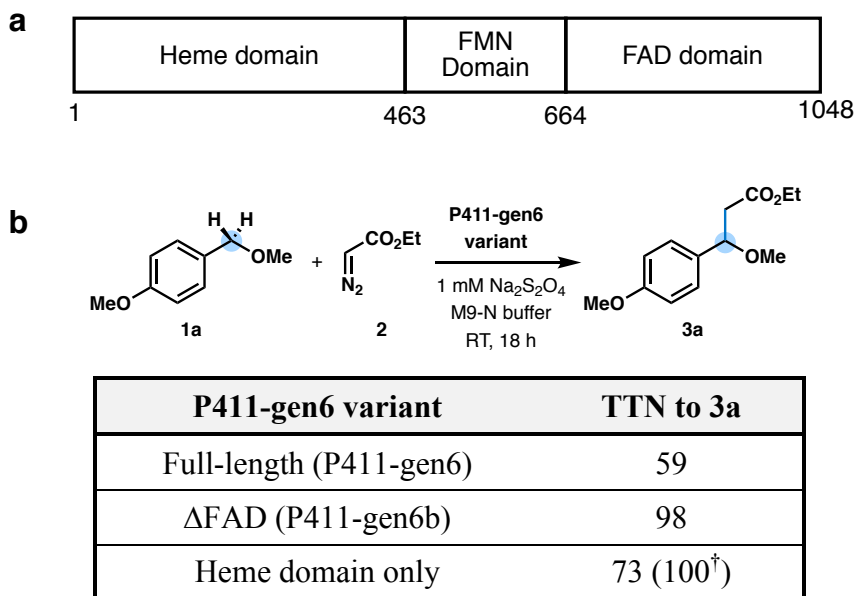
(K) Enzymatic reactions using purified heme protein. A solution of NADPH or Na₂S₂O₄ in phosphate buffer (0.1 M potassium phosphate, pH 8.0) was degassed by sparging with argon for at least 30 minutes (reaction solution). Separately, a solution of *D*-glucose (250 mM in 0.1 M potassium phosphate, pH 8.0) was also degassed in the same manner. Crimp vials (2 mL) were each charged with the GOX oxygen depletion system (20 µL of a stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate, pH 8.0). After degassing was complete, all solutions, 2 mL crimp vials, and purified protein (in 0.1 M potassium phosphate, pH 8.0), kept on ice, were brought into the anaerobic chamber. *D*-Glucose (40 µL of 250 mM solution), reaction buffer containing reductant (300 µL, reductant is either NADPH or Na₂S₂O₄), and purified protein (20 µL) were added to the 2 mL vial. The mixture was shaken for 5 min to ensure even distribution of the proteins; then, reaction vials were charged with alkane substrate (10 µL of 400 mM stock solution in EtOH) and ethyl diazoacetate (10 µL of 400 mM stock solution in EtOH). Final reaction volume was 400 µL. Final concentrations were 10 mM alkane substrate, 10 mM ethyl diazoacetate, and 25 mM *D*-glucose; concentrations of protein and reductant are variable and described with the individual experiment. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature and 500 rpm for 18 hours. The reactions were quenched by the addition of acetonitrile (400 µL) and internal standard (10 µL). This mixture was then transferred to a microcentrifuge tube and centrifuged (20,000 × g, 10 min). The supernatant was transferred to a vial and analyzed by HPLC.

II. Supplementary Figs. 1–11 and Supplementary Tables 1–5



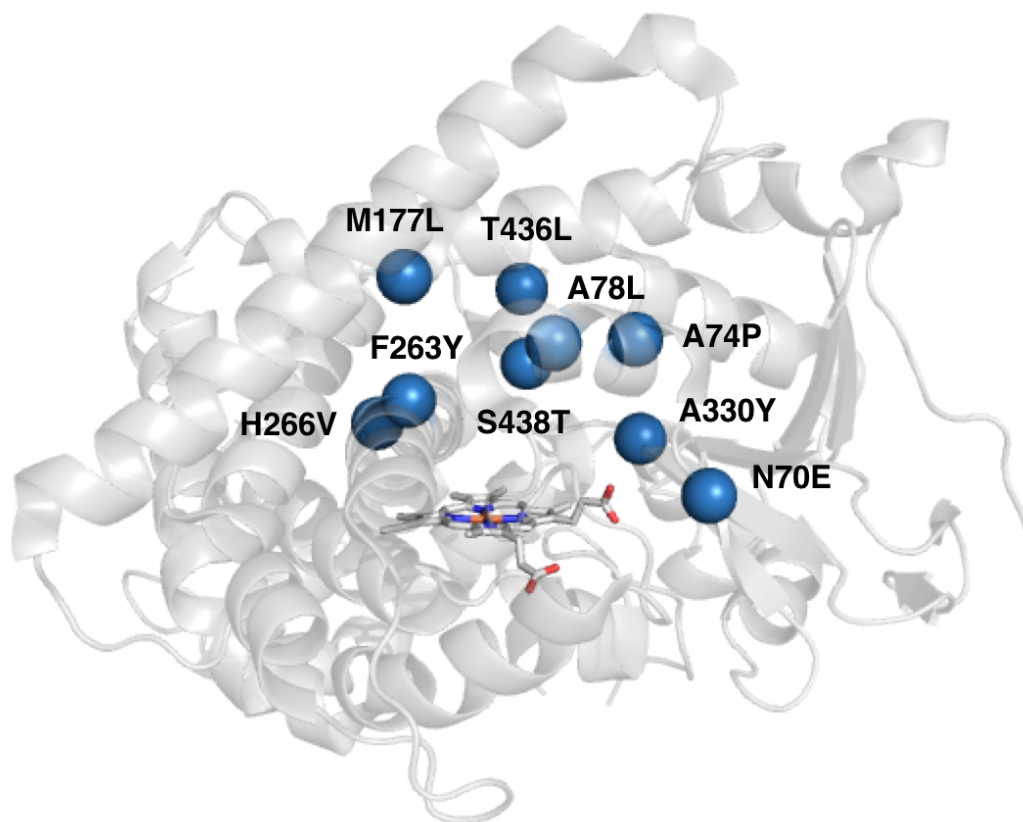
Supplementary Fig. 1. Evolutionary lineage from P-4 (A82L) to P411-CHF evaluated for C–H alkylation of 4-ethylanisole (**1i**).

Notes: Standard reaction conditions: clarified lysate of *E. coli* expressing the indicated heme protein variant (see **Section IV**), 10 mM substrate **1i**, 10 mM ethyl diazoacetate (**2**), 1 mM Na₂S₂O₄. Reactions with generations 1, 2, and 3 variants employed 4.0 μ M heme protein; all other reactions used 2.0 μ M heme protein. Bars represent mean TTN values averaged over four reactions (performed from two independent cell cultures, each used for duplicate reactions); each TTN data point is shown as a grey dot. Enantioselectivity results are represented by green diamonds. See Supplementary Table 13 in **Section IV** for the data presented here. TTN, total turnover number; RT, room temperature.



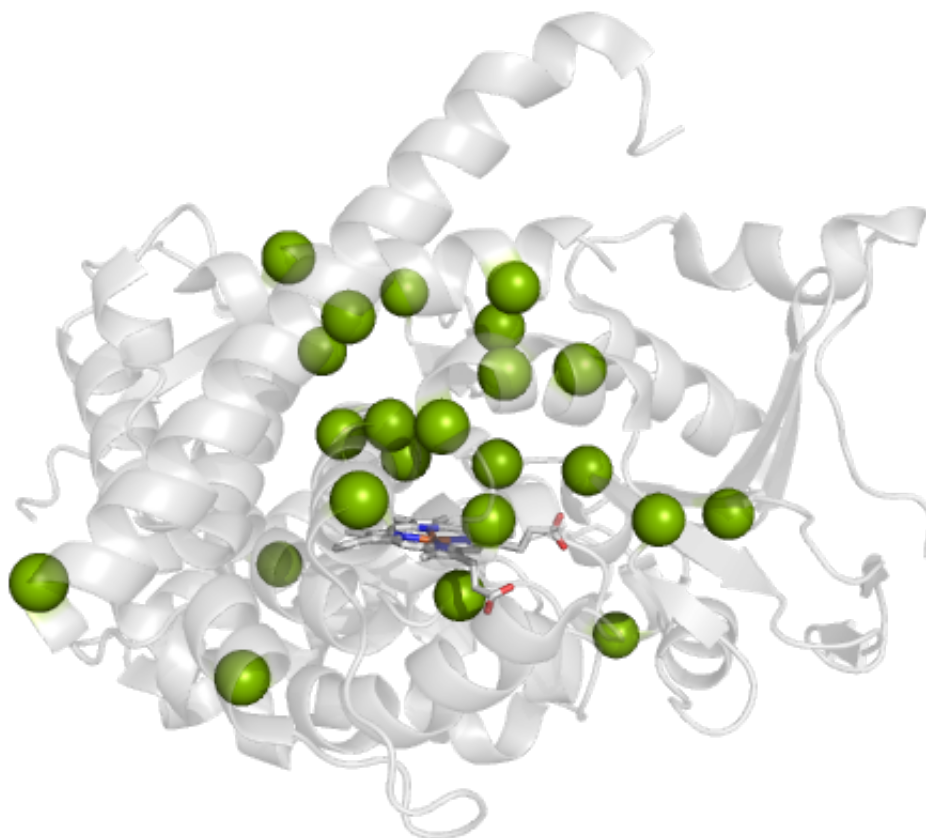
Supplementary Fig. 2. Truncation of a full-length P411 protein delivers active C–H alkylation enzymes.

Notes: **a**, Domain architecture of cytochrome P450_{BM3}. For its native monooxygenase activity, the FMN and FAD domains, collectively called the reductase domain, are responsible for delivering the necessary reducing equivalents from NADPH to the heme domain. The end of the FMN domain and the fragment of the polypeptide chain included in the ΔFAD complex were chosen based on a report by S. Govindaraj and T. L. Poulos⁵. **b**, Systematic truncation of the P411-gen6 full-length protein was performed to deliver P411-gen6b (P411ΔFAD-gen6, amino acids 1–664) and P411-gen6 heme-domain only (amino acids 1–463). Under the stated reaction conditions, both P411-gen6b (ΔFAD) and the heme domain only protein delivered **3a** with higher total turnover compared with the full-length protein. Standard reaction conditions: lysate of *E. coli* with 2.0 μM heme protein, 10 mM **1a**, 10 mM **2**, and 1 mM Na₂S₂O₄ (unless otherwise indicated). TTN results are an average of at least duplicate reactions. RT, room temperature; TTN, total turnover number. See **Section IV** for more information on P411-gen6 and P411-gen6b. [†]5 mM dithionite was used in these reactions.



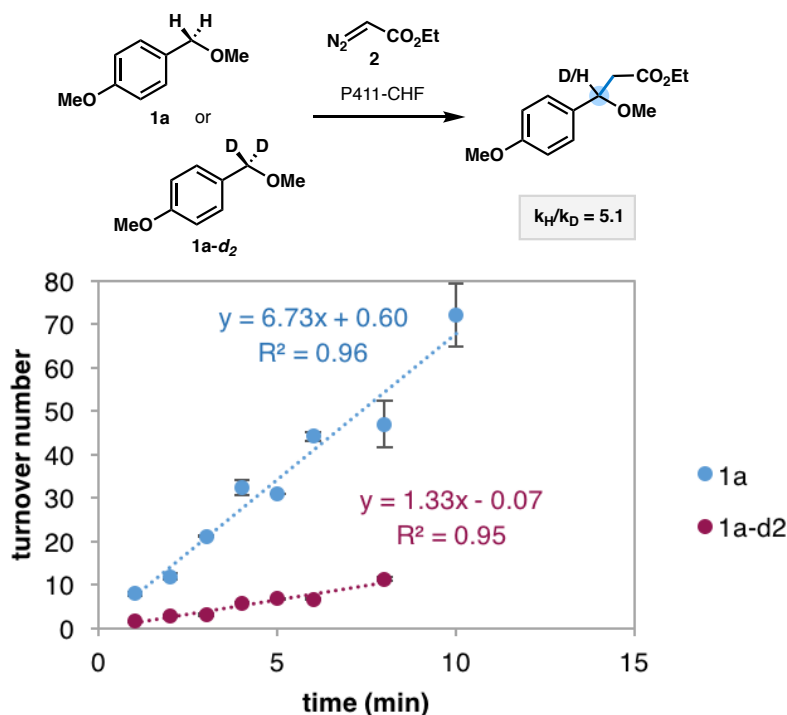
Supplementary Fig. 3. Structural visualization of amino acids mutated during directed evolution of P-4 (A82L) to P411-CHF.

Notes: The structure of P-4 (A82L) (heme domain) was modeled using the crystal structure of a related P411 variant (PDB 5UCW), which contains two additional mutations. Considering only the changes incurred in the heme domain, the following mutations were accumulated in going from P-4 (A82L) to P411-CHF: N70E, A74P, A78L, M177L, F263Y, H266V, A330Y, T436L, S438T (shown as blue spheres, residues 327 and 437 were not included in this analysis because P-4 (A82L) and P411-CHF contain the same amino acid residues at those positions). Most of the mutations are at positions that line the distal heme pocket and all of the mutated residues are within 15 Å of the iron atom in the heme cofactor.



Supplementary Fig. 4. Structural visualization of amino acid differences between P450_{BM3} wild-type and P411-CHF.

Notes: The structure of P450_{BM3} wild-type (heme domain) was modeled using the crystal structure of H. M. Girvan *et al.* (PDB 2IJ2). Comparing only the heme domains of the two proteins, the following 23 amino acids are changed in P411-CHF relative to P450_{BM3} wild-type: N70E, A74P, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, A328V, A330Y, L353V, I366V, C400S, T436L, E442K (shown as green spheres). Overall, 5% of the amino acids in the heme domain of P450_{BM3} have been substituted in P411-CHF.



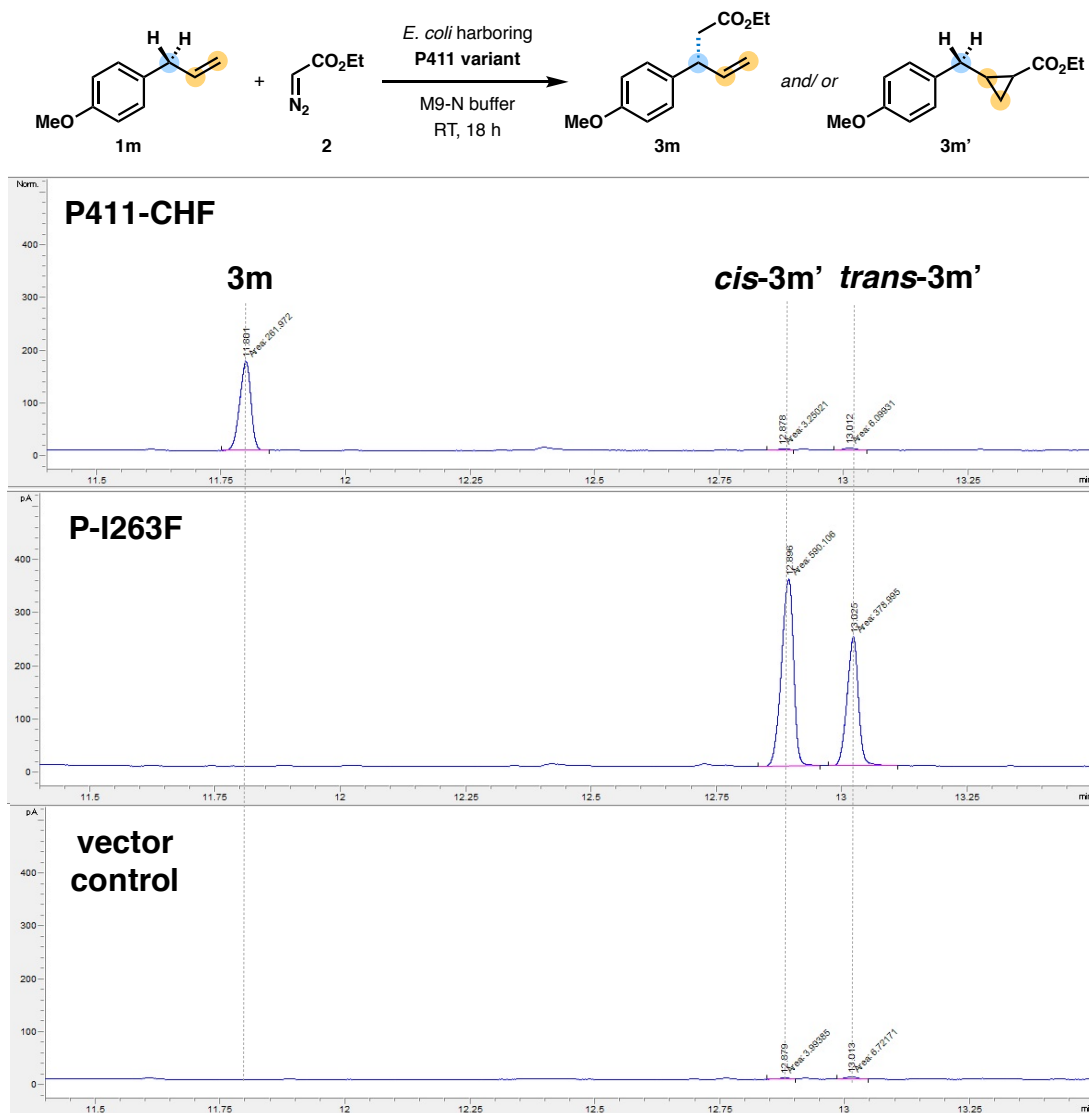
Supplementary Fig. 5. Kinetic isotope effect of C–H alkylation catalyzed by P411-CHF.

Notes: Data points represent an average of duplicate measurements; error bars represent one standard deviation. Data collected at the 10-minute time point using substrate **1a-d₂** were excluded due to non-linear behavior. Detailed experimental methods are described below.

Independent rate experiments with P411-CHF show an intermolecular kinetic isotope effect (KIE, k_H/k_D) of 5.1. This suggests that C–H insertion is rate-determining and could possibly involve a linear transition state⁶. In contrast, kinetic isotope effects for rhodium catalysts with carboxylate ligands are significantly less ($KIE = 1.55\text{--}3.2$)^{7,8}; this has been invoked as evidence to support a widely accepted three-centered transition state for C–H insertion with these systems⁹. The difference in KIE between P411-CHF and the rhodium-carboxylate catalysts suggests that these systems may have different transition states or different mechanisms for the C–H insertion step. Since the nature of the C–H insertion step could influence the substrate and product profiles of the catalyst, this is one strong motivation to develop diverse systems for this chemistry.

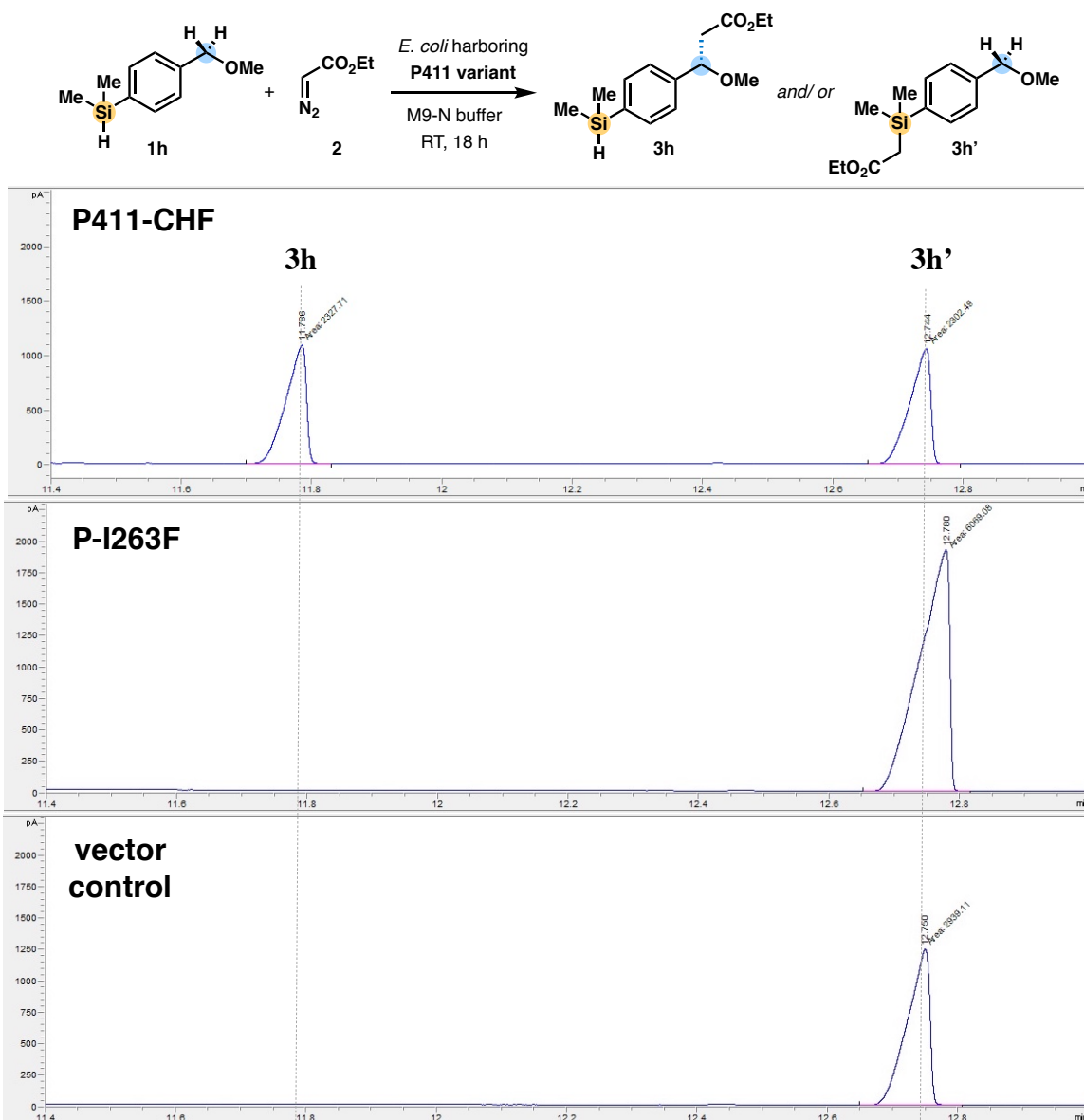
Determination of initial rates. Initial rates were measured from independent reactions set up in parallel using clarified lysate of *E. coli* cells overexpressing P411-CHF. The concentration of P411-CHF was normalized to be 2.0 μM in each reaction. A modified version of the procedure for reactions with lysate (**Section I (I)**) was followed. The modification is as follows: after combining all components of the reaction mixture except the alkane and diazo substrates, the 2 mL reaction vial was allowed to shake in the anaerobic chamber at 500 rpm for at least 10 minutes to ensure even mixing. Reaction vials were then charged with alkane substrate (10 μL , 400 mM in EtOH) and ethyl diazoacetate (10 μL , 400 mM in EtOH) and shaken at 500 rpm, room temp. Final concentrations were 2.0 μM P411-CHF, 1 mM $\text{Na}_2\text{S}_2\text{O}_4$, 10 mM alkane substrate, 10 mM ethyl diazoacetate, and 25 mM *D*-glucose. Reactions were set up in duplicate and quenched at the indicated time points by addition of acetonitrile (400 μL) and internal standard (10 μL , 60 mM ethyl phenoxyacetate in MeCN). This mixture was then removed from the anaerobic chamber,

transferred to a microcentrifuge tube, and centrifuged ($20,000 \times g$, 10 minutes). The supernatant was transferred to a vial and analyzed by HPLC (see **Section VIII**). Turnover number was calculated by dividing the concentration of product (mM) by concentration of P411-CHF (0.002 mM).



Supplementary Fig. 6. Product profiles for the reaction of 4-allylanisole (**1m**) and ethyl diazoacetate (**2**) with P411-CHF and with P-I263F.

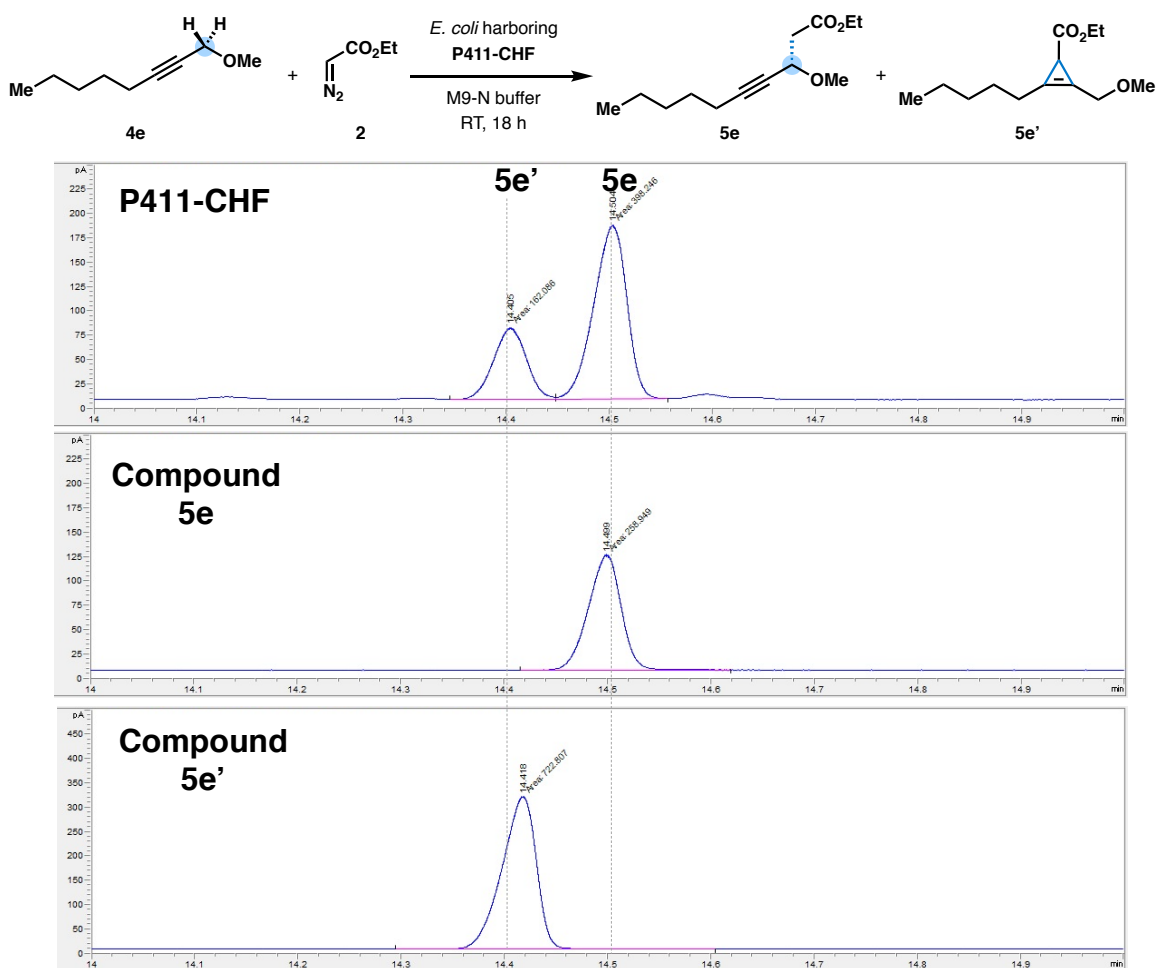
Notes: Representative GC traces of reactions performed with P411-CHF (top), P-I263F (middle), or a vector control (bottom) in *E. coli* cells. Reactions were performed with 10 mM substrate **1m** and 10 mM **2**, following the general procedure detailed in **Section I (H)**. Vector control indicates that *E. coli* harboring pET22b(+) encoding a protein that does not contain a transition metal cofactor (halohydrin dehalogenase, UniProt ID: Q93D82) was employed in the reaction.



Supplementary Fig. 7. P411-CHF catalyzes C–H alkylation of **1h** with ethyl diazoacetate (**2**).

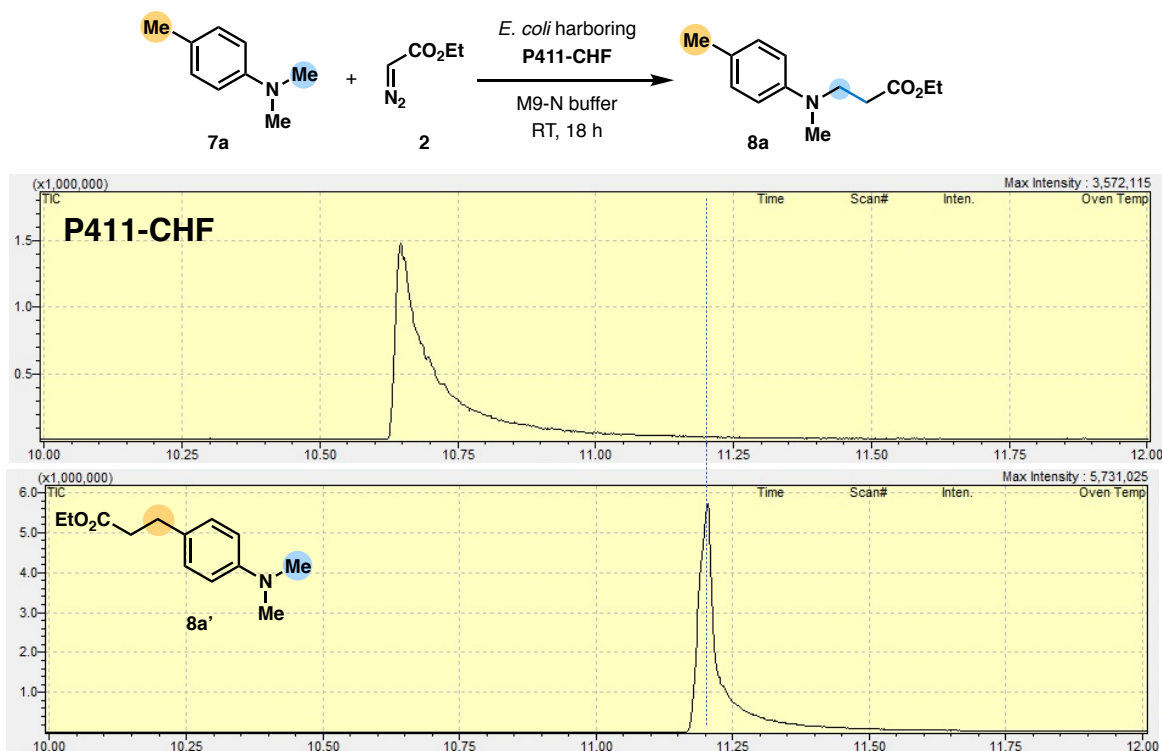
Notes: Representative GC traces of reactions performed with P411-CHF (top), P-I263F (middle), or a vector control (bottom) in *E. coli* cells. Reactions were performed as in **Supplementary Fig. 6**, with substrates **1h** and **2**.

While P411-CHF was able to access C–H alkylation product **3h** (930 TTN to **3h**, 1:1 **3h**:**3h'**) in the presence of a reactive Si–H bond, reaction with *E. coli* harboring P-I263F only afforded Si–H insertion product **3h'**. Additionally, compound **3h'** was also observed in vector control reactions employing whole *E. coli* cells. Neither **3h** nor **3h'** were detected in a control reaction where **1h** and **2** were combined in M9-N buffer in the absence of cells. See **Section VIII** for detailed analysis of product concentrations.



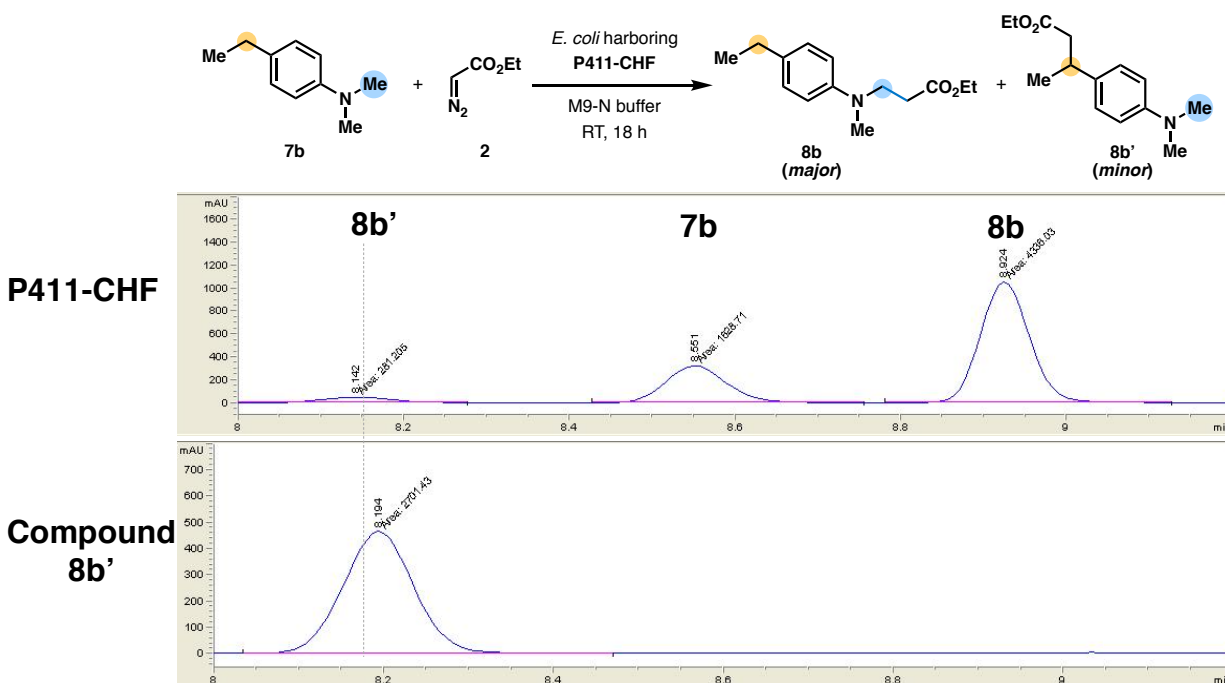
Supplementary Fig. 8. Enzymatic reaction of P411-CHF with substrate **4e** and ethyl diazoacetate (**2**) produces cyclopropene **5e'** as a side product.

Notes: Representative GC trace of a reaction performed using P411-CHF in *E. coli* cells (top) with 10 mM substrate **4e** and 10 mM ethyl diazoacetate (**2**), following the general procedure detailed in **Section I (H)**. Also shown are GC traces for reference compounds **5e** (middle) and **5e'** (bottom), used for the identification of P411-CHF synthesized products.



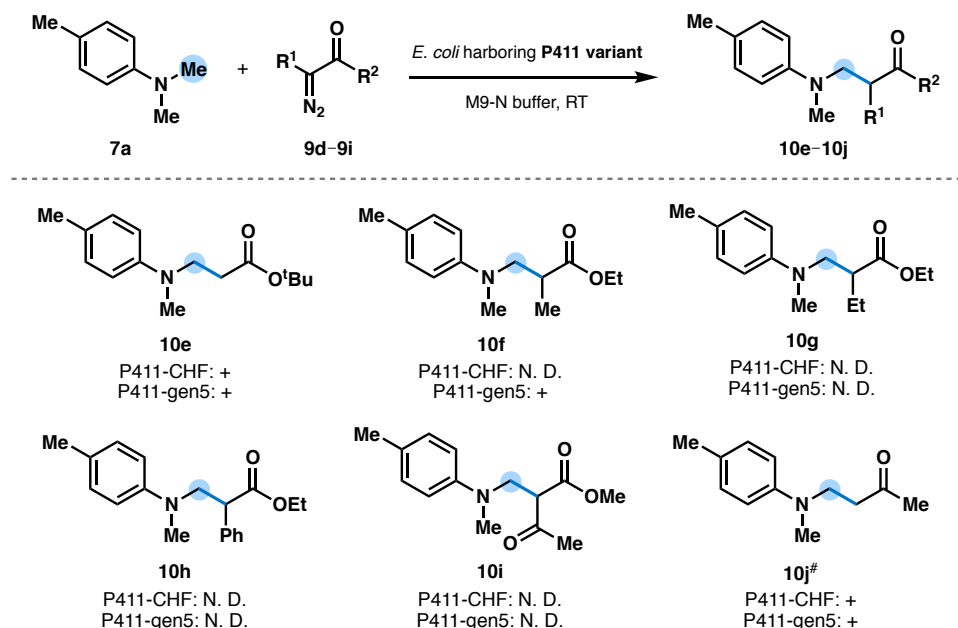
Supplementary Fig. 9. Enzymatic C–H alkylation of 4,*N,N*-trimethylaniline (**7a**) with P411-CHF is selective for the α -amino position.

Notes: Representative GC-MS trace of a reaction performed using P411-CHF in *E. coli* cells (top) with 10 mM substrate **7a** and 10 mM ethyl diazoacetate (**2**), following the general procedure detailed in **Section I (H)**. Also shown is a trace for reference compound **8a'** (bottom), which is not observed in the reaction with P411-CHF. This demonstrates that P411-CHF has exquisite selectivity for the alkylation of α -amino C–H bond in the presence of primary benzylic C–H bonds.



Supplementary Fig. 10. Product profile of P411-CHF catalyzed C–H alkylation of 4-ethyl-*N,N*-dimethylaniline (**7b**) with ethyl diazoacetate (**2**).

Notes: Representative HPLC trace of a reaction performed using P411-CHF in *E. coli* cells (top) with 10 mM substrate **7b** and 10 mM ethyl diazoacetate (**2**), following the general procedure detailed in **Section I (H)**. Also shown is a trace for reference compound **8b'** (bottom), which was identified as a minor side product in the P411-CHF catalyzed reaction. Major product **8b** was assigned by performing a preparative scale enzymatic reaction (see **Section IX**) and isolation and characterization of the major product.



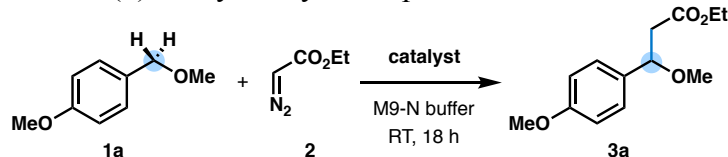
Supplementary Fig. 11. Additional diazo substrates tested for C–H alkylation with P411-CHF and P411-gen5. ‘+’ indicates product was detected; N. D., not detected. [#]Other products derived from compound **7a** were also observed by GC-MS.

Notes: Reactions were performed using *E. coli* cells expressing P411-CHF (resuspended to OD₆₀₀ = 59) or P411-gen5 (resuspended to OD₆₀₀ = 49) with 10 mM coupling partner **7a** and 10 mM diazo compound. Product formation was analyzed by GC-MS only. The identity of the product was not confirmed by comparison with chemically synthesized reference compounds or through isolation and characterization. These preliminary results are noteworthy, but should not be used alone for drawing conclusions.

Diazo compounds **9a**, **9b**, and **9c**, for the formation of products **10a**, **10b**, and **10c**, were also investigated with P411-CHF (+, +, +, respective preliminary results) and P411-gen5 (+, N. D., +). Additional variants were tested for these transformations and subsequent preparative scale reactions for product isolation and characterization were pursued; these results are described in Fig. 4c.

Because P411-CHF and its related variants are fully genetically encoded and can be readily optimized by directed evolution, these enzymes can serve as starting points for the evolution of future variants. Some of these future variants will certainly surpass P411-CHF in C–H alkylation activity with alternative diazo reagents.

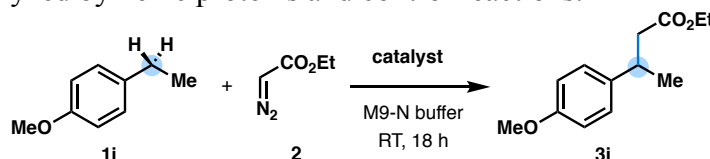
Supplementary Table 1. Initial results for C–H alkylation of *p*-methoxybenzyl methyl ether (**1a**) with ethyl diazoacetate (**2**) catalyzed by heme proteins and control reactions.



Catalyst	Catalyst concentration	TTN to 3a
P-4 (A82L) (in <i>E. coli</i> cells, OD ₆₀₀ = 29)	4.0 μM	13
<i>R. marinus</i> NOD (Y32G) (in <i>E. coli</i> cells, OD ₆₀₀ = 30)	11.6 μM	7 (12 [†])
Vector control (in <i>E. coli</i> cells, OD ₆₀₀ = 40) ^{*, ξ}	N. A.	n. d.
Hemin	400 μM	n. d. ^{†, #}
Hemin + BSA	400 μM (hemin), 0.6 mg/mL (BSA)	n. d. ^{†, #}

Notes: Reactions performed with 10 mM **1a** and 10 mM **2**; results are the average of duplicate reactions. BSA, bovine serum albumin; TTN, total turnover number; n. d., not detected. OD₆₀₀ value refers to that of the resuspension used for the reaction. [†]These reactions contain 1 mM Na₂S₂O₄, used as reductant. ^{*}Vector control indicates that *E. coli* harboring pET22b(+) encoding a protein which does not have a transition metal cofactor (halohydrin dehalogenase, UniProt ID: Q93D82) was employed in the reaction. ^ξReaction time was 24 h. [#]For reactions with hemin ± BSA, a 0.5 mM solution of hemin (± BSA, 0.75 mg/mL) in M9-N (320 μL) was used instead of a cell suspension; no *D*-glucose was added to these reactions.

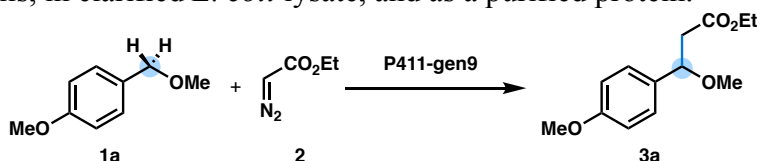
Supplementary Table 2. Initial results for C–H alkylation of 4-ethylanisole (**1i**) with ethyl diazoacetate (**2**) catalyzed by heme proteins and control reactions.



Catalyst	Catalyst concentration	TTN to 3i
P-4 (A82L) (in <i>E. coli</i> cells, OD ₆₀₀ = 21)	4.0 μM	3
<i>R. marinus</i> NOD (Y32G) (in <i>E. coli</i> cells, OD ₆₀₀ = 30)	11.6 μM	< 1 (2 [†])
Vector control (in <i>E. coli</i> cells, OD ₆₀₀ = 40) ^{*, ξ}	N. A.	n. d.
Hemin	400 μM	n. d. ^{†, #}
Hemin + BSA	400 μM (hemin), 0.6 mg/mL (BSA)	n. d. ^{†, #}

Notes: Reactions performed with 10 mM **1i** and 10 mM **2**; results are the average of duplicate reactions. Other notes are the same as in **Supplementary Table 1**.

Supplementary Table 3. P411-gen9, an evolved P411ΔFAD C–H alkylation enzyme, is active in whole *E. coli* cells, in clarified *E. coli* lysate, and as a purified protein.

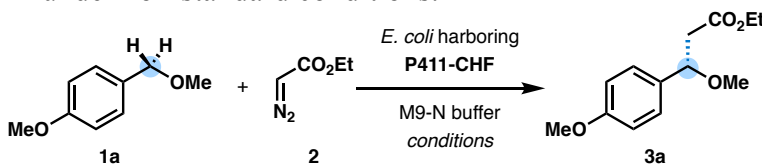


Form	[P411], μM	Exogenous reductant	TTN to 3a
whole <i>E. coli</i> cells (OD ₆₀₀ = 15–17)	2.0 μM	None	900
Lysate	2.0 μM	Na ₂ S ₂ O ₄ , 1 mM	940
Purified protein	11.9 μM	Na ₂ S ₂ O ₄ , 1 mM	150
Purified protein	11.9 μM	Na ₂ S ₂ O ₄ , 5 mM	210
Purified protein	10.0 μM –11.9 μM	NADPH, 10 mM	250

Notes: Reactions were performed with 10 mM **1a** and 10 mM **2** for 18–20 hours at room temperature, following the procedures detailed in **Section I (H, I, and K)**. Results are the average of at least two reactions. See **Section IV** for more information on P411-gen9.

These experiments demonstrate that C–H alkylation can be catalyzed using an evolved P411ΔFAD enzyme in whole *E. coli* cells, in clarified *E. coli* lysate, and as a purified protein. The results using purified P411-gen9 are preliminary and the TTN values should be interpreted with caution as no studies regarding the effect of purification conditions on the activity of the enzyme nor studies to optimize the reaction conditions were pursued.

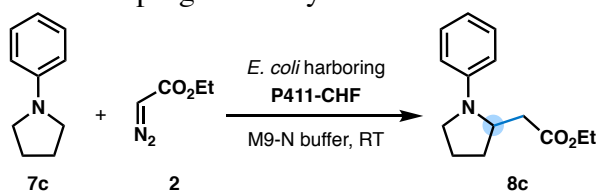
Supplementary Table 4. Enzymatic C–H alkylation reactions performed using *E. coli* cells harboring P411-CHF under non-standard conditions.



Conditions	TTN to 3a	e.r.
Anaerobic, full system, room temp.	2150	96.7 : 3.3
Anaerobic, full system, 4 °C	2090	98.0 : 2.0
Anaerobic, no GOX, room temp.	2100	96.7 : 3.3
Anaerobic, no GOX, no <i>D</i> -glucose, room temp.	1770	96.7 : 3.3
Aerobic, no GOX, no <i>D</i> -glucose, room temp.	30	not measured

Notes: Standard reaction conditions are *E. coli* cells expressing P411-CHF (resuspended to OD₆₀₀ = 30) with 10 mM **1a** and 10 mM ethyl diazoacetate (**2**). Reactions were allowed to proceed at the indicated temperature for 18 h. For reactions without the GOX oxygen depletion solution, 20 μL of M9-N buffer was added instead; for reactions without *D*-glucose, 40 μL of M9-N buffer was added. The aerobic reaction was set up under air, without prior degassing of cells or reagents with argon. Results are the average of duplicate reactions.

Supplementary Table 5. Reaction progress analysis of enantioselectivity.



Time	Relative amount of 8c formed [†]	e.r.
15 min	0.045	83.6 : 16.4
30 min	0.062	83.2 : 16.8
1 h	0.093	83.5 : 16.5
20 h	1	83.9 : 16.1
18 h (<i>isolated</i>) [‡]	not applicable	82.8 : 17.2

Notes: Standard reaction conditions are *E. coli* cells expressing P411-CHF (resuspended to OD₆₀₀ = 30) with 10 mM **7c** and 10 mM ethyl diazoacetate (**2**), following the general procedure detailed in **Section I (H)**. Reactions were quenched after the indicated time by the addition of cyclohexane (500 μ L) and internal standard (10 μ L of 40 mM ethyl benzoate). The mixture was transferred to a 1.5 mL microcentrifuge tube, vortexed (10 seconds, 3 times), and centrifuged (20,000 \times g, 5 minutes) to completely separate the organic and aqueous layers. The organic layer was taken for chiral HPLC analysis (see **Section XI**). [†]This refers to the ratio of the HPLC peak areas for **8c** (sum of enantiomers) over internal standard in a reaction quenched at the indicated time to that of a reaction quenched at 20 h. [‡]Product was isolated after an enzymatic reaction for 18 h; preparative scale reaction conditions were employed and full details are provided in **Section IX**.

This experiment demonstrates that enantioselectivity of enzymatic C–H alkylation does not change with time and substrate conversion. While it is possible that the protein is altered during the course of the reaction¹⁰, this does not affect the enantioselectivity of the product.

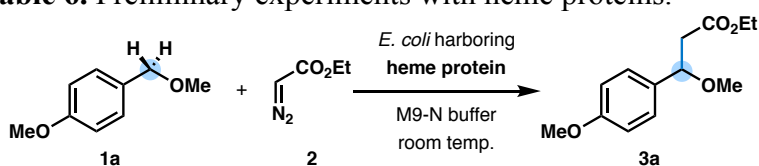
III. Screening of enzymes for C–H alkylation activity

Testing diverse heme proteins for reaction discovery

A composite plate of 40 heme proteins and their variants from various organisms were screened for formation of product **3a** from substrates **1a** and **2**. These proteins were cloned and used in other studies, including carbene Si–H insertion⁴ and alkene cyclopropanation^{11,12}. Expression of these proteins followed the procedures as described by the prior studies and testing for initial activity was carried out with whole *E. coli* cells. The general procedure for reaction screening in 96-well plate format (**Section I (E)**) was employed and the reactions were analyzed by GC-MS. *Note*: Since this was an initial test, proper expression of the indicated proteins was not verified. Consequently, negative results should be interpreted with caution. From this experiment, it was observed that *R. marinus* NOD (Y32G) had a low level of C–H alkylation activity (Supplementary Table 6).

In addition, eight diverse heme proteins, including *R. marinus* NOD (Y32G), were chosen and tested for C–H alkylation activity using substrates **1a** and **2** following the general procedure for small scale enzymatic reactions using whole *E. coli* cells (**Section I (H)**) (Supplementary Table 7). Product formation by variant *R. marinus* NOD (Y32G) was characterized by HPLC (see **Section VIII**). Other reactions were analyzed by GC-MS for formation of product **3a** and the expression of all proteins were measured using the hemochrome assay. Conditions with no exogenous reductant as well as with the addition of 1 mM Na₂S₂O₄ (final concentration) were tested.

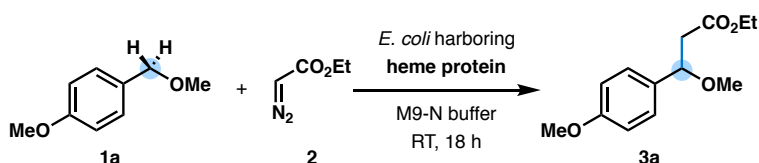
Supplementary Table 6. Preliminary experiments with heme proteins.



UniProt ID	Organism	Annotation	Abbrev. name	No. of variants	Ref.	Formation of 3a
B3FQS5	<i>Rhodothermus marinus</i>	Cytochrome <i>c</i>	<i>Rma</i> cyt <i>c</i>	9	4	n. d.
P15452	<i>Hydrogenobacter thermophilus</i>	Cytochrome <i>c</i> -552	<i>Hth</i> cyt <i>c</i>	2	4	n. d.
P00080	<i>Rhodopila globiformis</i>	Cytochrome <i>c</i> 2	-	2	4	n. d.
D0MGT2	<i>Rhodothermus marinus</i>	Nitric oxide dioxygenase	<i>Rma</i> NOD	10	11	detected [†]
P02185	<i>Physeter catodon</i> (Sperm whale)	Myoglobin	Mb	1	11*	n. d.
B3DUZ7	<i>Methylophilum infernorum</i>	Hemoglobin-like flavoprotein	HGG [#]	2	11*	n. d.
O66586	<i>Aquifex aeolicus</i>	Thermoglobin	-	1	11	n. d.
G7VHJ7	<i>Pyrobaculum ferrireducens</i>	Protoglobin	-	1	11	n. d.
Q0PB48	<i>Campylobacter jejuni</i>	Truncated hemoglobin	-	1	11	n. d.
Q9YFF4	<i>Aeropyrum pernix</i>	Protoglobin	-	10	11	n. d.
O31607	<i>Bacillus subtilis</i>	Truncated hemoglobin	-	1	12	n. d.

Notes: Reactions performed with 10 mM **1a** and 10 mM **2**. No., number; Abbrev., abbreviated; Ref., reference.; n. d., not detected. [†]Small amount of **3a** was detected in the reaction with variant *R. marinus* NOD (Y32G). No product **3a** was detected in the reaction mixtures of other *R. marinus* NOD variants. *While these heme proteins were not reported in this reference, the expression conditions employed for these variants followed those given in the reference. [#]This protein is abbreviated as Hell's Gate Globin (HGG) on the basis of a prior literature report which uses this name¹³.

Supplementary Table 7. Heme proteins tested for C–H alkylation activity.



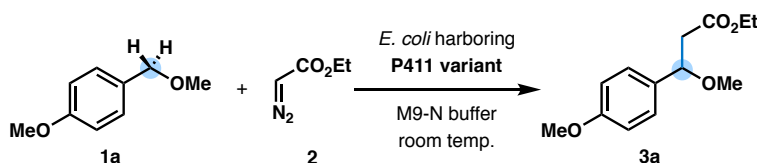
UniProt ID	Organism	Annotation	Abbrev. Name	Mutation(s) from WT	Formation of 3a
P14779	<i>Bacillus megaterium</i>	cytochrome P450/NADPH-P450 reductase	P450 _{BM3}	none [*]	n. d.
Q55080	<i>Sulfolobus acidocaldarius</i>	Cytochrome P450	CYP119	none [*]	n. d.
D0MGT2	<i>Rhodothermus marinus</i>	Nitric oxide dioxygenase	<i>Rma</i> NOD	Y32G ^{+, *}	7 TTN [†] (12 TTN ^ξ)
B3DUZ7	<i>Methylophilum infernorum</i>	Hemoglobin-like flavoprotein	HGG	none ^{+, *}	n. d.
P02185	<i>Physeter catodon</i> (Sperm whale)	Myoglobin	Mb	H64V V68A D122N ^{+, *}	n. d.
B3FQS5	<i>Rhodothermus marinus</i>	Cytochrome <i>c</i>	<i>Rma</i> cyt <i>c</i>	none ^{+, #}	n. d.
B3FQS5	<i>Rhodothermus marinus</i>	Cytochrome <i>c</i>	<i>Rma</i> cyt <i>c</i>	V75T M100D M103E ^{+, #}	n. d.
P15452	<i>Hydrogenobacter thermophilus</i>	Cytochrome <i>c</i>	<i>Hth</i> cyt <i>c</i>	none ^{+, #}	n. d.

Notes: Heme proteins were tested in whole *E. coli* cells (OD₆₀₀ = 30) both with and without the addition of 1 mM Na₂S₂O₄ (final concentration). Reactions were performed with 10 mM **1a** and 10 mM **2**; results are the average of at least duplicate reactions. Abbrev., abbreviated; WT, wild-type; n. d., not detected; TTN, total turnover number. [†]This data is also included in Supplementary Table 1. ^ξReaction includes 1 mM Na₂S₂O₄. ⁺These variants are also included in Supplementary Table 6. ^{*}These proteins were expressed following the procedure of A. M. Knight *et al.*¹¹. [#]Cytochrome *c* variants were expressed following the procedure of S. B. J. Kan *et al.*⁴.

Screening previously engineered cytochrome P450_{BM3} variants

A composite plate of 36 cytochrome P450_{BM3} variants from lineages engineered for non-natural reactions including C–H amination¹⁴, olefin aziridination¹⁵, and olefin cyclopropanation^{16a} was screened for C–H alkylation activity using substrates **1a** and **2**. Variants of cytochrome P450_{BM3} in which the axial cysteine ligand is replaced with serine are termed cytochrome P411^{16b}. The general procedure for reaction screening in 96-well plate format (**Section I (E)**) was employed and the reactions were analyzed by HPLC. Most variants showed no activity (17 variants) or trace activity (15 variants) for **3a** formation. The four highest performing variants, all which contain an axial cysteine-to-serine mutation (P411), are shown in Supplementary Table 8. While P-4 (A82L) was chosen as the parent protein for the directed evolution of a C–H alkylation enzyme, the information gained from this experiment was used to guide the first several rounds of evolution.

Supplementary Table 8. Engineered cytochrome P411 variants show promiscuous C–H alkylation activity.



Name	Mutations from P450 _{BM3} WT	Relative amount of 3a formed [†]
P-4 (A82L)	V78A, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K	1
P-4 (A82L A78V F263Y)	A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K	1.4
P-4 (A82L A78V F263L)	A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263L, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K	0.5
P-4 (A82L A78V F263L E267D) (a.k.a. P411 _{CHA})	A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263L, E267D, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K	0.7

Notes: Reactions were performed with 10 mM **1a** and 10 mM **2** following the general procedure for reaction screening in 96-well plate format (**Section I (E)**). Results are the average of duplicate reactions. All variants are reported in ref. 14. [†] This refers to the ratio of the HPLC peak area for **3a** in reactions with the indicated variant to that in reactions with P-4 (A82L).

IV. Directed evolution of C–H alkylation enzymes

Construction of site-saturation libraries

Site-saturation libraries were generated employing the “22c-trick” method¹ and screened in one 96-well plate; double site-saturation libraries were generated using the same method to target two different sites and these were screened in three 96-well plates. Following the general screening in 96-well plate procedure (**Section I (E)**), variants which exhibited higher formation of C–H alkylation product (**3a** or **3i**) or improved enantioselectivity for product **3a** were identified. A summary of the amino acid residues targeted for mutagenesis is presented in Supplementary Table 9, as well as the beneficial mutation(s) selected for each round of mutagenesis. The locations of the selected beneficial mutations are displayed on a structural model of the P411 enzyme (**Section II**, Supplementary Fig. 3).

Variants which were identified to show higher activity and/ or enantioselectivity during screening were streaked out on LB_{amp} agar plates. A single colony was selected, sequenced, and the TTN measured for both products **3a** and **3i** using clarified lysate of *E. coli* cells overexpressing the desired protein (unless otherwise indicated, the concentration of P411 variant was normalized such that each reaction contained 2.0 μ M enzyme, general procedure **Section I (I)**). Enantiomeric ratios of the enzymatic products produced by P411-gen6 and further evolved variants were also characterized. The results are summarized in Supplementary Tables 12 and 13.

Supplementary Table 9. Summary of directed evolution for C–H alkylation.

Round	Parent	Diversification strategy [†]	Screening substrate (selection criteria)	Changes made [*]
1	P-4 (A82L)	Individual variants identified as active for C–H amination ¹⁴	4-ethylanisole (1i) (activity)	F263Y
2	P-4 (A82L F263Y)	Site-saturation mutagenesis A78X	4-ethylanisole (1i) (activity)	A78L
3	P-4 (A82L F263Y A78L)	Site-saturation mutagenesis T327X	4-ethylanisole (1i) (activity)	T327I
4	P411-gen4 (P-4 (A82L F263Y A78L T327I))	Site-saturation mutagenesis A74X, E267X	4-ethylanisole (1i) (activity)	A74G
5	P411-gen5 (P411-gen4 (A74G))	Site-saturation mutagenesis A328X, H92X, R255X, A264X, H100X, F393X, L437X	<i>p</i> -methoxybenzyl methyl ether (1a) (activity)	L437Q
6	P411-gen6 (P411-gen5 (L437Q))	Protein truncations: full-length P411, Δ FAD domain, heme-domain only	<i>p</i> -methoxybenzyl methyl ether (1a) (activity)	Δ FAD domain
6b	P411-gen6b	Site-saturation mutagenesis	<i>p</i> -methoxybenzyl	S438T

	(P411ΔFAD-gen6)	A78X, F87X, I263X, T438X	methyl ether (1a) (activity)	
7	P411-gen7 (P411-gen6b (S438T))	Site-saturation mutagenesis ^ψ A330X, F331X, T436X, A82X, L181X, L188X	<i>p</i> -methoxybenzyl methyl ether (1a) (activity)	T436L
8	P411-gen8 (P411-gen7 (T436L))	Site-saturation mutagenesis D63X, F162X, M177X, V178X, L439X	4-ethylanisole (1i) (activity)	M177L
9	P411-gen9 (P411-gen8 (M177L))	Site-saturation mutagenesis F87X, E267X, M118X, L437X, H266X, S332X, T260X, T365X	4-ethylanisole (1i) (activity)	H266V
10	P411-gen10 (P411-gen9 (H266V))	Site-saturation mutagenesis N70X, T88X, H171X, H361X, P329X, T269X, L75X, L52X	4-ethylanisole (1i) (activity) & <i>p</i> -methoxybenzyl methyl ether (1a) (enantioselectivity)	N70E
11	P411-gen11 (P411-gen10 (N70E))	Site-saturation mutagenesis L71X, S72X, F261X, G265X, L86X, I401X, A330X, C400X	<i>p</i> -methoxybenzyl methyl ether (1a) (activity & enantioselectivity)	A330Y
12	P411-gen12 (P411-gen11 (A330Y))	Double site-saturation mutagenesis P329X-F331X [#] , A328X-F331X [#] , T327X-T268X, A74X-L437X	<i>p</i> -methoxybenzyl methyl ether (1a) (activity & enantioselectivity)	I327T
13a	P411-gen13 (P411-gen12 (I327T))	Double site-saturation mutagenesis L181X-L437X, V178X-E267X, M118X-I401X	<i>p</i> -methoxybenzyl methyl ether (1a) (activity & enantioselectivity)	None
13b	P411-gen13 (P411-gen12 (I327T))	Testing previously identified beneficial and neutral mutations ^ξ .	<i>p</i> -methoxybenzyl methyl ether (1a) (activity & enantioselectivity)	G74P, Q437L
14	P411-CHF (P411-gen13 (G74P Q437L))	N. A.	N. A.	N. A.

Notes: Some residues were saturated more than once, in different parents. Gen, generation; N. A., not applicable. [†]Residues for site-saturation mutagenesis libraries are listed relative to the amino acid at that position in wild-type P450_{BM3}. ^{*}Beneficial mutations are listed relative to the amino

acid at that position in the parent protein. ^ψRandom mutagenesis by error-prone PCR on this parent enzyme and screening for C–H alkylation activity was also performed (unpublished results). However, no mutations from this study were carried forward to the next enzyme generation; the F162L mutation, identified in this context, was included in the diversification strategy of round 13b. [#]Only NDT libraries were constructed and screened for this double-site saturation experiment.

^εSelect mutations identified from previous rounds of mutagenesis and screening were introduced in various combinations to P411-gen13. Twenty-seven variants were attempted and screened.

Supplementary Table 10. Mutations and truncations relative to P450_{BM3} wild-type for the P411 variants described in Supplementary Table 9.

Gen.	Name	Mutations relative to P450 _{BM3} WT	Domain composition [†]
1	P-4 (A82L)	V78A, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K	Full-length
2	P-4 (A82L F263Y)	V78A, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K	Full-length
3	P-4 (A82L F263Y A78L)	V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, A328V, L353V, I366V, C400S, T438S, E442K	Full-length
4	P411-gen4 (P-4 (A82L F263Y A78L T327I))	V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T438S, E442K	Full-length
5	P411-gen5 (P411-gen4 (A74G))	A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T438S, E442K	Full-length
6	P411-gen6 (P411-gen5 (L437Q))	A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, L437Q, T438S, E442K	Full-length
6b	P411-gen6b (P411ΔFAD–gen6)	A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, L437Q, T438S, E442K	ΔFAD domain
7	P411-gen7 (P411-gen6b (S438T))	A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, L437Q, E442K	ΔFAD domain
8	P411-gen8 (P411-gen7 (T436L))	A74G, V78L, A82L, F87A, P142S, T175I, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T436L, L437Q, E442K	ΔFAD domain

9	P411-gen9 (P411-gen8 (M177L))	A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T436L, L437Q, E442K	Δ FAD domain
10	P411-gen10 (P411-gen9 (H266V))	A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T436L, L437Q, E442K	Δ FAD domain
11	P411-gen11 (P411-gen10 (N70E))	N70E, A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, T327I, A328V, L353V, I366V, C400S, T436L, L437Q, E442K	Δ FAD domain
12	P411-gen12 (P411-gen11 (A330Y))	N70E, A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, T327I, A328V, A330Y, L353V, I366V, C400S, T436L, L437Q, E442K	Δ FAD domain
13	P411-gen13 (P411-gen12 (I327T))	N70E, A74G, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, A328V, A330Y, L353V, I366V, C400S, T436L, L437Q, E442K	Δ FAD domain
14	P411-CHF (P411-gen13 (G74P Q437L))	N70E, A74P, V78L, A82L, F87A, P142S, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, A328V, A330Y, L353V, I366V, C400S, T436L, E442K	Δ FAD domain

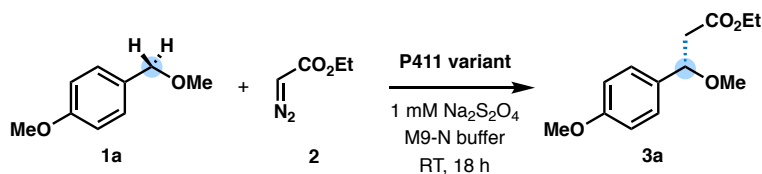
[†]Full-length cytochrome P411 variants contain amino acids 1–1048. Cytochrome P411 variants containing the FAD truncation (Δ FAD domain) contain amino acids 1–664.

Supplementary Table 11. Other P411 variants described in this study.

Name	Mutations from P450 _{BM3} WT	Reference
P-I263F	V78A, F87V, P142S, T175I, A184V, S226R, H236Q, E252G, I263F, T268A, A290V, L353V, I366V, C400S, T438S, E442K	17
P411-IY (T327I)	N70E, A74G, V78L, A82L, F87A, M118S, P142S, F162L, T175I, M177L, A184V, S226R, H236Q, E252G, I263Y, H266V, T268G, A290V, T327I, A328V, A330Y, L353V, I366V, C400S, I401L, T436L, L437Q, E442K	N. A. [†]

[†]N. A., not applicable. The parent of this variant, P411-IY, was made for round 13b of directed evolution (see Supplementary Table 9). The P411-IY (T327I) variant, which contains an additional T327I mutation, was identified in the context of a separate carbene transfer project (Chen, K., Zhou, A. Z. & Arnold, F. H., unpublished results).

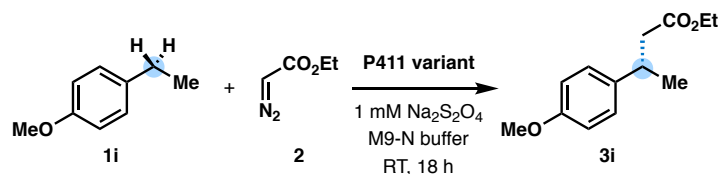
Supplementary Table 12. Enzymatic C–H alkylation data presented in Fig. 2b.



Variant	[P411], μ M	TTN \pm SD	e.r.
P-4 (A82L)	4.0	14 \pm 2	N. A.
P-4 (A82L F263Y)	4.0	29 \pm 1	N. A.
P-4 (A82L F263Y A78L)	4.0	23 \pm 5	N. A.
P411-gen4	2.0	48 \pm 3	N. A.
P411-gen5	2.0	68 \pm 3	N. A.
P411-gen6	2.0	59 \pm 4	<i>rac</i>
P411-gen6b	2.0	98 \pm 2	<i>rac</i>
P411-gen7	2.0	200 \pm 4	55.0 : 45.0
P411-gen8	2.0	560 \pm 50	59.4 : 40.6
P411-gen9	2.0	940 \pm 30	64.7 : 35.3
P411-gen10	2.0	1480 \pm 50	68.1 : 31.9
P411-gen11	2.0	1240 \pm 30	77.9 : 22.1
P411-gen12	2.0	1490 \pm 40	88.5 : 11.5
P411-gen13	2.0	1960 \pm 40	94.0 : 6.0
P411-CHF	2.0	2020 \pm 40	96.7 : 3.3

Notes: Reactions were performed using clarified *E. coli* lysate with 10 mM **1a** and 10 mM **2**, following the general procedure detailed in **Section I (I)**. Each reported TTN is the average over four reactions (performed from two independent cell cultures, each used for duplicate reactions). TTN, total turnover number, refers to TTN to **3a**; SD, standard deviation; N. A., not applicable, indicates that this value was not measured; *rac*, racemic. Parts of this data are also included in Supplementary Fig. 2 and Supplementary Table 3.

Supplementary Table 13. Enzymatic C–H alkylation data presented in Supplementary Fig. 1.



Variant	[P411], μ M	TTN \pm SD	e.r.
P-4 (A82L)	4.0	2 \pm 0	N. A.
P-4 (A82L F263Y)	4.0	4 \pm 0	N. A.
P-4 (A82L F263Y A78L)	4.0	7 \pm 2	N. A.
P411-gen4	2.0	13 \pm 1	N. A.
P411-gen5	2.0	14 \pm 0	N. A.
P411-gen6	2.0	12 \pm 1	N. A.
P411-gen6b	2.0	18 \pm 1	N. A.
P411-gen7	2.0	34 \pm 1	N. A.
P411-gen8	2.0	130 \pm 20	67.7 : 32.3
P411-gen9	2.0	260 \pm 20	71.9 : 28.1
P411-gen10	2.0	510 \pm 30	76.9 : 23.1
P411-gen11	2.0	450 \pm 10	83.0 : 17.0
P411-gen12	2.0	630 \pm 20	92.0 : 8.0
P411-gen13	2.0	500 \pm 30	96.9 : 3.1
P411-CHF	2.0	440 \pm 30	98.0 : 2.0

Notes: Reactions were performed using clarified *E. coli* lysate with 10 mM **1i** and 10 mM **2**, following the general procedure detailed in **Section I (I)**. Each reported TTN is the average over four reactions (performed from two independent cell cultures, each used for duplicate reactions). TTN, total turnover number, refers to TTN to **3i**; SD, standard deviation; N. A., not applicable, indicates that this value was not measured.

V. Nucleotide and amino acid sequences

All heme proteins disclosed below were cloned into a pET22b(+) vector and contain a C-terminal 6xHis-tag.

DNA and amino acid sequence of **P-4 (A82L)**, a previously reported cytochrome P411 variant¹⁴ which was used as the starting point for directed evolution:

```
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DNA and amino acid sequence of **P411-gen5**, which was employed for the synthesis of key intermediate (+)-**8f** in the total synthesis of (+)-cuspareine:

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DNA and amino acid sequence of P411-CHF, an evolved C–H alkylation enzyme:

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DNA and amino acid sequence of **P411-IY (T327I)**, a C–H alkylation enzyme derived from P411-gen13.

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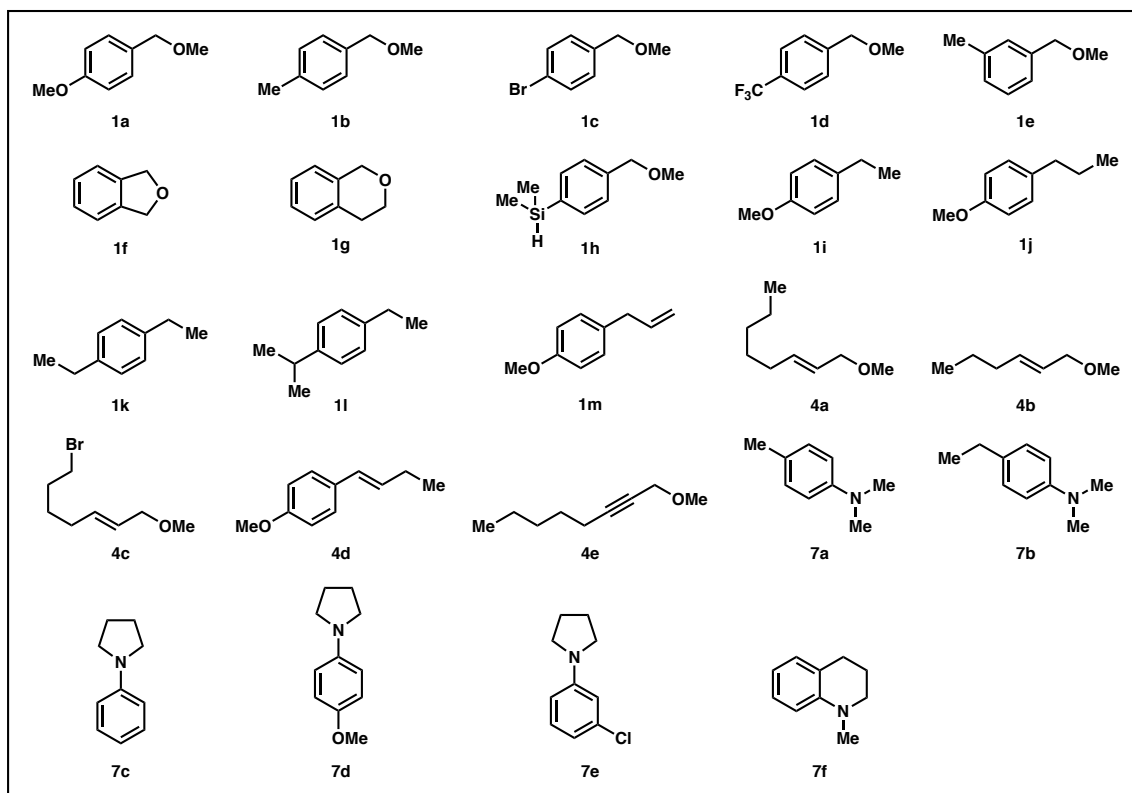
DNA and amino acid sequence of *R. marinus* NOD (Y32G), a heme protein which exhibits trace C–H alkylation activity.

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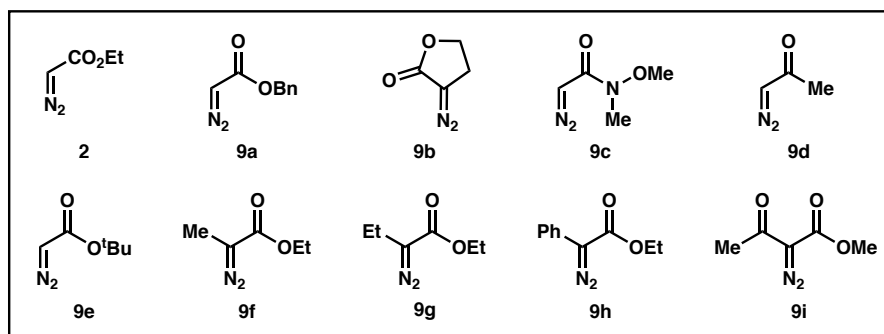
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VI. Substrate Synthesis and Characterization

Commercially available alkane and diazo substrates were used as received: **1a**, **1d**, **1f**, **1g**, **1i–1k**, **1m**, **7a**, **7c–7f**, **9a**, **9e**, **9f** (custom synthesis, Arch Bioscience). Compound **1c** was also commercial (Combi-Blocks), though the commercial product was used only for synthesis. Ethyl diazoacetate (**2**, Sigma-Aldrich) was concentrated under reduced pressure and its concentration relative to residual dichloromethane was determined by ^1H NMR. Diazo compounds **9h** (ref. 18) and **9i** (ref. 19) are known and were prepared according to literature procedures. Caution: although no safety issues were encountered, diazo compounds are reactive and should be used with caution.



Supplementary Fig. 12. Alkane substrates. This terminology is used to emphasize that the reaction reported here acts on a sp^3 -hybridized C–H bond in the alkane portion of the substrate.



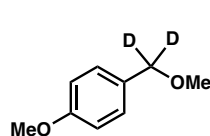
Supplementary Fig. 13. Diazo compounds.

General Procedure A: Methylation of alcohols

To a 250 mL round bottom flask was added NaH (60% dispersion in mineral oil, 15–30 mmol, 1.2–1.5 equiv.). The flask was evacuated and filled with argon (3 times). Anhydrous THF (45–80 mL) was added by syringe and the reaction mixture was cooled to 0 °C in an ice bath. Alcohol (10–20 mmol, 1.0 equiv.) in THF (5–10 mL) was added dropwise and the reaction mixture was allowed to warm to room temperature and stirred for 30 minutes. Following, iodomethane (20–40 mmol, 2.0 equiv.) in THF (10 mL) was added and the reaction was stirred at room temperature (8–15 hours). The reaction was quenched by the addition of brine (60 mL) or NH₄Cl (sat. aq., 60 mL) and the phases were separated. The aqueous layer was extracted with diethyl ether (3 × 60 mL); the combined organics were washed with aq. sodium thiosulfate (10% w/v, 50 mL, when necessary), dried over Na₂SO₄ and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate or pentane / diethyl ether afforded compounds the desired products in 37–99% yield.

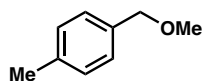
1-Methoxy-4-(methoxymethyl-*d*₂)benzene (1a-*d*₂)

Labeled substrate **1a-*d*₂** was prepared from methyl 4-methoxybenzoate using a two-step sequence to 98% deuterium incorporation at the benzylic position. First, to a dry round bottom flask, under argon, was added LiAlD₄ (0.23 g, 5.5 mmol, 1.1 equiv.) and anhydrous Et₂O (10 mL). A solution of methyl 4-methoxybenzoate (0.83 g, 5 mmol, 1.0 equiv.) in dry Et₂O (5 mL) was added dropwise and the reaction was allowed to stir at room temperature for 12 hours. Following, the reaction mixture was cooled to 0 °C and diluted with Et₂O. The reaction was quenched by the addition of 0.2 mL H₂O, 0.2 mL NaOH (aq., 1M), and 0.6 mL H₂O. The mixture was allowed to warm to room temperature and stirred for 15 minutes. MgSO₄ was added and the mixture was stirred for a further 15 minutes, filtered, and concentrated under reduced pressure. The crude product was purified by silica column chromatography with hexanes / ethyl acetate to give (4-methoxyphenyl)methanol-*d*₂ (0.43 g, 61% yield, 98% deuterium incorporation), with spectral data in agreement with literature report²⁰. Methylation of this compound was performed following **General Procedure A** (note: reaction performed on 3.0 mmol scale) to afford **1a-*d*₂** (0.43 g, 61% yield, 98% deuterium incorporation).



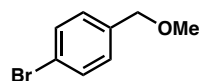
¹H NMR (400 MHz, CDCl₃) δ 7.27 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 3.81 (s, 3H), 3.36 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 159.3, 130.3, 129.5, 113.9, 73.7 (m, labeled), 57.8, 55.4. HRMS (EI) *m/z*: 154.0964 (M⁺); calc. for C₉H₁₀O₂²H₂: 154.0963.

1-(Methoxymethyl)-4-methylbenzene (1b)



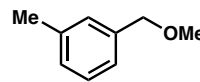
Prepared from *p*-tolylmethanol using **General Procedure A**. This compound is known in the literature²¹. ¹H NMR (400 MHz, CDCl₃) δ 7.23 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 7.9 Hz, 2H), 4.43 (s, 2H), 3.37 (s, 3H), 2.35 (s, 3H).

1-Bromo-4-(methoxymethyl)benzene (1c)



Prepared from (4-bromophenyl)methanol using **General Procedure A**. This compound is known²¹. **¹H NMR** (400 MHz, CDCl₃) δ 7.47 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.6 Hz, 2H), 4.41 (s, 2H), 3.38 (s, 3H).

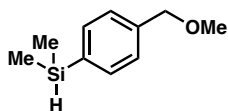
1-(Methoxymethyl)-3-methylbenzene (1e)



Prepared from *m*-tolylmethanol using **General Procedure A**. **¹H NMR** (400 MHz, CDCl₃) δ 7.28 – 7.21 (m, 1H), 7.19 – 7.08 (m, 3H), 4.43 (s, 2H), 3.40 (s, 3H), 2.36 (s, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 138.2, 128.6, 128.5, 128.4, 124.9, 74.9, 58.3, 21.5. **HRMS** (FAB) m/z : 135.0810 [(M + H⁺)–H₂]; calc. for C₉H₁₁O: 135.0810.

(4-(Methoxymethyl)phenyl)dimethylsilane (1h)

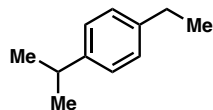
In a 250 mL round bottom flask, under argon, 1-bromo-4-(methoxymethyl)benzene (3.0 g, 15 mmol, 1.0 equiv.) in anhydrous THF (60 mL) was cooled to -78 °C. A solution of *n*-butyllithium (9 mL, 2.5 M in hexanes, 22.5 mmol, 1.5 equiv.) was added dropwise. The resulting mixture was stirred at -78 °C for 2 hours before the dropwise addition of chlorodimethylsilane (2.4 mL, 22.5 mmol, 1.5 equiv.). The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was cooled to 0 °C and quenched with NH₄Cl (sat. aq., 20 mL). The aqueous layer was extracted with diethyl ether (3 × 30 mL); the combined organics were washed with brine (30 mL), dried over Na₂SO₄ and concentrated under reduced pressure. The crude reaction mixture was purified by silica column chromatography with hexanes / ethyl acetate to afford **1h** (2.14 g, 79% yield). A second round of purification by silica column chromatography with hexanes / ether was performed on a portion of the product.



¹H NMR (400 MHz, CDCl₃) δ 7.54 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 4.47 (s, 2H), 4.43 (hept, J = 3.7 Hz, 1H), 3.40 (s, 3H), 0.35 (d, J = 3.7 Hz, 6H). **¹³C NMR** (101 MHz, CDCl₃) δ 139.3, 136.9, 134.3, 127.3, 74.7, 58.3, -3.6. **HRMS** (FAB) m/z : 179.0894 [(M + H⁺)–H₂]; calc. for C₁₀H₁₅OSi: 179.0892.

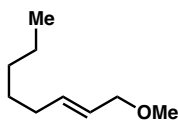
1-Ethyl-4-isopropylbenzene (1l)

The following procedure was modified from the literature²². To a 250 mL round bottom flask were added Pd/C (10% Pd on activated charcoal, 486 mg, 20% w/w), 4-isopropylacetophenone (2.43 g, 15 mmol), and methanol (60 mL). The solution was sparged with H₂ and stirred under 1 atm H₂ for 48 hours; monitoring the mixture by TLC showed that the reaction did not go to completion under these conditions. The crude reaction mixture was filtered through a pad of Celite, dried over Na₂SO₄, and concentrated under reduced pressure. Purification by silica column chromatography with hexanes afforded product **1l** (218 mg, 1.47 mmol, 10% yield).



$^1\text{H NMR}$ (500 MHz, CDCl_3) δ 7.19 – 7.13 (m, 4H), 2.90 (hept, $J = 6.9$ Hz, 1H), 2.65 (q, $J = 7.6$ Hz, 2H), 1.29 – 1.24 (m, 9H). $^{13}\text{C NMR}$ (126 MHz, CDCl_3) δ 146.2, 141.7, 127.9, 126.5, 33.8, 28.5, 24.2, 15.7. **HRMS** (FAB) m/z : 149.1327 ($\text{M} + \text{H}^+$); calc. for $\text{C}_{11}\text{H}_{17}$: 149.1330.

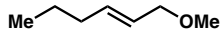
(*E*)-1-Methoxyoct-2-ene (4a)



Prepared from (*E*)-oct-2-en-1-ol using **General Procedure A**. This compound is known in the literature²³. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.70 (dt, $J = 15.6$, 6.6, 1.2 Hz, 1H), 5.54 (dt, $J = 15.3$, 6.2, 1.4 Hz, 1H), 3.86 (dq, $J = 6.2$, 1.0 Hz, 2H), 3.31 (s, 3H), 2.08 – 1.99 (m, 2H), 1.43 – 1.34 (m, 2H), 1.34 – 1.21 (m, 4H), 0.88 (t, $J = 7.0$ Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 135.2, 126.1, 73.5, 57.8, 32.4, 31.5, 28.9, 22.7, 14.2.

(*E*)-1-Methoxyhex-2-ene (4b)

Prepared from (*E*)-hex-2-en-1-ol using a modified version of **General Procedure A**. To a 100 mL dry round bottom flask, cooled under argon, were added (*E*)-hex-2-en-1-ol (2.0 g, 20 mmol, 1.0 equiv.), DMF (35 mL), and iodomethane (5.7 g, 40 mmol, 2.0 equiv.). The resulting solution was cooled to 0 °C and NaH (60% dispersion in mineral oil, 960 mg, 24 mmol, 1.2 equiv.) was added portion-wise. The mixture was stirred at 0 °C for 30 minutes, then allowed to warm to room temperature and stirred for an additional 3 hours. The reaction mixture was cooled to 0 °C, quenched with the addition of NH_4Cl (sat. aq., 30 mL), and diluted with diethyl ether (50 mL). Phases were separated and the aqueous layer was extracted with diethyl ether (3 \times 50 mL). The combined organics were washed with H_2O (2 \times 25 mL) and brine (25 mL), dried over Na_2SO_4 , and concentrated under reduced pressure (≥ 200 mbar). Purification by silica column chromatography with pentane / diethyl ether afforded compound **4b** (746 mg, 6.5 mmol, 33% yield).

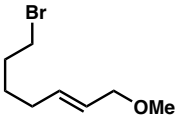


This compound is known in the literature²⁴. $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 5.70 (dt, $J = 15.4$, 6.6, 1.2 Hz, 1H), 5.55 (dt, $J = 15.4$, 6.3, 1.4 Hz, 1H), 3.87 (dq, $J = 6.3$, 1.1 Hz, 2H), 3.32 (s, 3H), 2.06 – 2.00 (m, 2H), 1.42 (app. sext, $J = 7.4$ Hz, 2H), 0.91 (t, $J = 7.3$ Hz, 3H). $^{13}\text{C NMR}$ (126 MHz, CDCl_3) δ 134.9, 126.3, 73.4, 57.8, 34.5, 22.4, 13.8.

(*E*)-7-Bromo-1-methoxyhept-2-ene (4c)

To a 100 mL flamed dried flask was added Grubbs' catalyst 2nd generation (85 mg, 1 mol%). The flask was then evacuated and backfilled with argon for three times. Under argon, a dry CH_2Cl_2 solution containing 6-bromo-1-hexene (1.63 g, 10 mmol, 1.0 equiv.) and crotonaldehyde (3.50 g, 50 mmol, 5.0 equiv.) was added to the flask. The mixture was stirred under reflux for 20 hours and then cooled to room temperature and filtered through a silica plug. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (hexanes / ethyl acetate) to give (*E*)-7-bromohept-2-enal (1.6 g, 84% yield). This product was then dissolved in 10 mL dry THF and then added slowly to a suspension of NaBH_4 (375 mg, 10 mmol, 1.0 equiv.) in dry THF (10 mL) at 0 °C. To this reaction mixture, iodine (1.27 g, 5 mmol, 0.5 equiv.) in 10 mL of THF was slowly added at 0 °C. Reaction was stirred until the

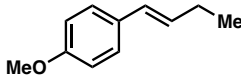
aldehyde was fully reduced as indicated by TLC. The reaction was quenched with NH_4Cl (sat. aq.), the phases were separated, and the aqueous phase was extracted with ethyl acetate (3×20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4 . The solvent was removed under reduced pressure and the crude alcohol product was used directly without purification. **General Procedure A** was used for the methylation step and the final product **4c** was obtained with 50% overall yield (1.03g, 5 mmol).

 This compound is known in the literature²⁵. **^1H NMR** (400 MHz, CDCl_3) δ 5.68 (dt, $J = 15.3, 6.4, 1.1$ Hz, 1H), 5.57 (dt, $J = 15.4, 6.0, 1.2$ Hz, 1H), 3.86 (dq, $J = 5.9, 1.0$ Hz, 2H), 3.41 (t, $J = 6.8$ Hz, 2H), 3.32 (s, 3H), 2.14 – 2.05 (m, 2H), 1.92 – 1.82 (m, 2H), 1.57 – 1.49 (m, 2H). **^{13}C NMR** (101 MHz, CDCl_3) δ 133.9, 127.0, 73.3, 57.9, 33.8, 32.3, 31.5, 27.7. **HRMS** (EI) m/z : 205.0216 ($M - \text{H}^+$); calc. for $\text{C}_8\text{H}_{14}^{79}\text{BrO}$: 205.0228.

(*E*)-1-(But-1-en-1-yl)-4-methoxybenzene (**4d**)

This compound was accessed in a two-step sequence. First, to propyltriphenylphosphonium bromide (7.6 g, 19.7 mmol, 1.0 equiv.) suspended in anhydrous THF (70 mL) and cooled to 0 °C was added *n*-butyllithium (2.5 M in hexanes, 7.9 mL, 19.7 mmol, 1.0 equiv.) dropwise over 10 min to form a bright orange solution. After stirring for 1 hour, 4-methoxybenzaldehyde (2.7 g, 19.7 mmol, 1.0 equiv.) was added dropwise over 5 min. The reaction mixture was allowed to slowly warm to room temperature and stirred at room temperature overnight. The reaction mixture was diluted with pentane (50 mL) and the resulting solution was washed with HCl (aq., 0.1 M, 50 mL), H_2O (50 mL), NaHCO_3 (sat. aq., 50 mL), and brine (50 mL). The organics were dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with pentane / diethyl ether to afford (*E:Z*)-**4d** (2:1 *E:Z*, 2.50 g, 15.4 mmol, 78% yield).

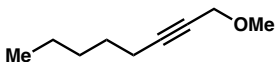
Next, (*E:Z*)-**4d** was isomerized following a literature method²⁶. To a dry 25 mL round bottom flask, under argon, were added (*E:Z*)-**4d** (300 mg, 1.85 mmol), $(\text{MeCN})_2\text{PdCl}_2$ (235 mg, 50 mol%), and 4 mL anhydrous dichloromethane. The resulting mixture was stirred at room temperature for 24 hours. The crude reaction mixture was filtered through Celite and concentrated under reduced pressure. Purification by silica column chromatography using hexanes / diethyl ether delivered **4d** (> 20:1 *E:Z*, 279 mg, 1.72 mmol, 93% yield).

 This compound is known in the literature²⁶. **^1H NMR** (400 MHz, CDCl_3) δ 7.28 (d, $J = 8.7$ Hz, 2H), 6.85 (d, $J = 8.8$ Hz, 2H), 6.33 (dt, $J = 15.7, 1.6$ Hz, 1H), 6.13 (dt, $J = 15.8, 6.5$ Hz, 1H), 3.80 (s, 3H), 2.26 – 2.16 (m, 2H), 1.08 (t, $J = 7.5$ Hz, 3H). **^{13}C NMR** (101 MHz, CDCl_3) δ 158.7, 130.9, 130.7, 128.2, 127.1, 114.0, 55.4, 26.2, 14.0.

1-Methoxyoct-2-yne (**4e**)

To a solution of 3-methoxyprop-1-yne (845 μL , 10 mmol, 1.0 equiv.) in anhydrous THF (50 mL) at -20 °C, was added *n*-butyllithium (2 M in THF, 6 mL, 12 mmol, 1.2 equiv.) and HMPA (869 μL , 5 mmol, 0.5 equiv.) dropwise over 5 min. The resulting mixture was stirred at -20 °C for 3 hours before the addition of 1-iodopentane (1.96 mL, 15 mmol, 1.5 equiv.). The reaction was allowed to slowly warm to room temperature in 2 hours and stirred for additional 18 hours. The

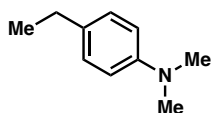
reaction was then quenched by NH_4Cl (sat. aq., 20 mL) and H_2O (30 mL), and extracted by diethyl ether (40 mL \times 3). The combined organic layer was washed by H_2O (50 mL) and brine (50 mL), and then dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with pentane / diethyl ether to afford **4e** (1.04 g, 7.4 mmol, 74% yield).



This compound is known in the literature²⁷. **^1H NMR** (400 MHz, CDCl_3) δ 4.07 (t, J = 2.2 Hz, 2H), 3.37 (s, 3H), 2.22 (tt, J = 7.2, 2.2 Hz, 2H), 1.56 – 1.47 (m, 2H), 1.41 – 1.26 (m, 4H), 0.89 (t, J = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, CDCl_3) δ 87.4, 75.8, 60.4, 57.5, 31.2, 28.5, 22.3, 18.9, 14.1. **HRMS** (EI) m/z : 139.1128 [$(\text{M} - \text{H})^+$]; calc. for $\text{C}_9\text{H}_{15}\text{O}$: 139.1123.

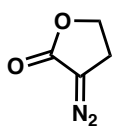
4-Ethyl-*N,N*-dimethylaniline (**7b**)

4-Ethylaniline (0.605 g, 5 mmol, 1.0 equiv.) and formaldehyde (1.8 mL, 50 mmol, 10.0 equiv.) were mixed in acetic acid (30 mL). The solution was stirred for 30 min at 0 °C before portionwise addition of NaBH_3CN (1.57 g, 25 mmol, 5.0 equiv.). After the reaction was stirred overnight, NaOH (aq., 2M) was used to neutralize the reaction at 0 °C until pH 8-10. The crude product was extracted with diethyl ether (30 mL \times 3). The combined organic layer was washed with H_2O (50 mL) and brine (50 mL), and then dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with hexanes / ethyl acetate to afford **7b** (635 mg, 4.25 mmol, 85% yield).



This compound is known in the literature²⁸. **^1H NMR** (400 MHz, CDCl_3) δ 7.09 (d, J = 8.8 Hz, 2H), 6.72 (d, J = 8.7 Hz, 2H), 2.92 (s, 6H), 2.57 (q, J = 7.6 Hz, 2H), 1.21 (t, J = 7.6 Hz, 3H). **^{13}C NMR** (101 MHz, CDCl_3) δ 149.1, 132.8, 128.5, 113.3, 41.2, 27.9, 16.1.

3-Diazodihydrofuran-2(3*H*)-one (**9b**)



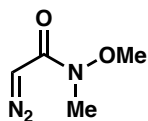
The preparation of the title compound **9b** followed a modified procedure reported by Sattely *et al*²⁹. Sodium azide (4.83 g, 74.3 mmol, 4 equiv.), sodium hydroxide (80 mL of 2 M in water, 160 mmol), tetrabutylammonium bromide (60.0 mg, 0.190 mmol, 0.01 equiv.), and hexane (80 mL) were combined in a 500-mL flask with magnetic stir bar open to the air and cooled to 0 °C. With vigorous stirring, triflic anhydride (6.20 mL, 37.1 mmol, 2 equiv.) was added dropwise. After 15 min, a solution of 2-acetyl-butylolactone (2.00 mL, 18.6 mmol) in acetonitrile (70 mL) was poured into the vessel through a funnel, followed by an additional 10 mL of acetonitrile to complete the transfer. The initially colorless reaction mixture immediately turned yellow. After stirring for 20 min at 0 °C, the mixture was diluted with ice water (50 mL) and chilled EtOAc (50 mL) and transferred to a separatory funnel. After phase separation and removal of the organic fraction, the aqueous layer was washed with chilled EtOAc (50 mL \times 5). The combined organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The resulting crude product was purified by silica column chromatography with hexanes / ethyl acetate as eluents. The yellow-colored fractions were concentrated to afford the product as a bright yellow crystalline solid (1.2–1.6 g, 60–75% yield). Spectral data are consistent with Sattely *et al*²⁹.

2-Diazo-*N*-methoxy-*N*-methylacetamide (9c)

4-Methylbenzenesulfonohydrazide (9.31 g, 50 mmol, 1.0 equiv.) was dissolved in aqueous hydrochloric acid (2 M, 30 mL) and warmed to 50 °C (solution 1). 2-Oxoacetic acid (7.40 g of 50% in water, 50 mmol, 1.0 equiv.) was dissolved in water (100 mL) and heated to 50 °C (solution 2). Pre-warmed solution 1 was slowly transferred to solution 2. The reaction mixture was then stirred at 60 °C for 4 h until all the hydrozone product crashed out. The mixture was cooled to 0 °C and kept for 2 h. The product 2-(2-tosylhydrazineylidene)acetic acid (9.88 g, 82% yield) was collected by filtration, washed with hexane: ethyl acetate (10:1) and dried under vacuum.

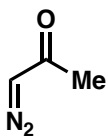
2-(2-Tosylhydrazineylidene)acetic acid (4.84 g, 20 mmol, 1.0 equiv.) was dissolved in dry dichloromethane (30 mL). Thionyl chloride (16 mL) and *N,N*-dimethyl formaldehyde (3 drops, *cat.*) were added to the solution. The reaction mixture was stirred at room temperature for 1 h and then heated to reflux (~ 50 °C) for 5 h until the starting material was completely dissolved and the reaction turned clear and light yellow. After the reaction was cooled to room temperature, organic solvent and the excess thionyl chloride was removed under reduced pressure. The resulting mixture (solid) was then dissolved in dry dichloromethane (20 mL) and used for the next step.

N,O-Dimethylhydroxylamine hydrochloride (3.91 g, 40 mmol, 2.0 equiv.) and triethylamine (11.2 mL, 80 mmol, 4.0 equiv.) were mixed in dry dichloromethane (80 mL) and stirred for 30 min. The solution of acyl chloride was added dropwise over 20 min to the reaction mixture at 0 °C. The reaction was then stirred at room temperature for 5 h before water (80 mL) was added to quench the reaction. The liquid phases were transferred to a separatory funnel, and the aqueous phase was extracted with dichloromethane (50 mL × 4). The combined organic phase was washed with water (40 mL) and brine (40 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resulting crude product was purified by silica column chromatography with hexanes / ethyl acetate to afford **9c** as a yellow liquid (0.82 g, 32% yield).



This compound is known in the literature³⁰. ¹H NMR (400 MHz, CDCl₃) δ 5.33 (s, 1H), 3.66 (s, 3H), 3.19 (s, 3H).

1-Diazopropan-2-one (9d)

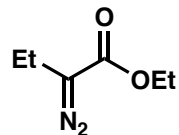


The preparation of the title compound **9d** followed a modified procedure reported by Zhang *et al*³¹. To a solution of acetylacetone (3.4 mL, 33.0 mmol, 1.10 equiv.) and triethylamine (5.04 mL, 36.4 mmol, 1.21 equiv.) in dry acetonitrile (25 mL), a solution of *p*-acetamidobenzenesulfonyl azide (7.20 g, 30.0 mmol, 1.0 equiv.) in dry acetonitrile (25 mL) was added dropwise. The reaction mixture was stirred at room temperature for 4 h. Then, the solvent was removed under reduced pressure and the resulting mixture was then purified by silica column chromatography with hexanes / ethyl acetate to give 3-diazopentane-2,4-dione (3.65 g, 96% yield) as a pale yellow liquid.

3-Diazopentane-2,4-dione (1.89 g, 15 mmol, 1.0 equiv.) was dissolved in diethyl ether (25 mL). An aqueous solution (25 mL) of NaOH (3.00 g, 75 mmol, 5.0 equiv.) was added dropwise over 10 min to the ether layer with vigorous stirring at 0 °C. The reaction mixture turned dark brown within 20 min and was then stirred at room temperature for 4 h. The liquid phases were transferred to a separatory funnel, and the aqueous phase was extracted with diethyl ether (30 mL × 5). The combined organic phase was dried over Na₂SO₄, filtered, and concentrated under reduced

pressure ($T = 24\text{ }^{\circ}\text{C}$, $P \geq 20\text{ kPa}$) to give product **9d** as a volatile yellow liquid (0.892 g, 71% yield). Spectral data is consistent with Zhang *et al*³¹.

Ethyl 2-diazobutanoate (**9g**)



The preparation of the title compound **9g** followed a modified procedure reported by Huang *et al*³². To a solution of ethyl 2-ethylacetoacetate (3.16 g, 20.0 mmol, 1.0 equiv.) and *p*-acetamidobenzenesulfonyl azide (7.21 g, 30.0 mmol, 1.5 equiv.) in dry acetonitrile (50 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 4.5 mL, 30.0 mmol, 1.5 equiv.) dropwise at $0\text{ }^{\circ}\text{C}$. The reaction mixture was stirred at $0\text{ }^{\circ}\text{C}$ for 1 h and at room temperature for 2 h. Water (50 mL) was added to quench the reaction. Acetonitrile was removed under reduced pressure ($T = 24\text{ }^{\circ}\text{C}$, $P \geq 20\text{ kPa}$). The mixture was extracted with diethyl ether (25 mL \times 4). The combined ether layer extract was washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure ($T = 24\text{ }^{\circ}\text{C}$, $P \geq 30\text{ kPa}$). The crude product was purified by silica column chromatography with hexanes / ethyl acetate to give product **9g** as a volatile yellow liquid (2.40 g, 84% yield). Spectral data is consistent with Huang *et al*³².

VII. Synthesis and characterization of reference compounds

Racemic reference compounds corresponding to enzymatic products and side-products were prepared according to the following procedures. Reference compounds are characterized below.

General Procedure B: Aldol reaction and methylation synthetic sequence

In a dry 100 or 250 mL round bottom flask, under argon, a solution of diisopropylamine (6–24 mmol, 1.1–1.2 equiv.) in THF (15–30 mL) was cooled to 0 °C (**General Procedure B-1**) or -78 °C (**General Procedure B-2**). *n*-Butyllithium (6–25 mmol, 1.1–1.2 equiv., 1.6 or 2.5 M in hexanes) was added dropwise and the resulting mixture was stirred at the appropriate temperature for 15–30 min. The mixture was cooled to -78 °C and kept at this temperature for the remainder of the reaction. Ethyl acetate (14–28 mmol, 1.4 equiv., **General Procedure B-1** or 6–10 mmol, 1.0 equiv., **General Procedure B-2**) was added dropwise and the mixture was stirred for an additional 30–45 min. Then, aldehyde (10–20 mmol, 1.0 equiv., **General Procedure B-1** or 9–11 mmol, 1.1–1.5 equiv., **General Procedure B-2**) as a solution in THF (15–30 mL, **General Procedure B-1**) or neat (**General Procedure B-2**) was added slowly and the solution was stirred for a further 0.5–3 hours. The reaction mixture was quenched at -78 °C by the addition of NH₄Cl (sat. aq., 10–30 mL) and allowed to thaw to room temperature. For **General Procedure B-1** only, HCl (1 M aq., 1.5–3.0 mL) was also added. Phases were separated and the aqueous phase was extracted with ethyl acetate or diethyl ether (3 × 20–30 mL). The combined organics were washed with NH₄Cl (sat. aq., 2 × 10–15 mL), brine (10 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded the desired aldol adducts in 56–95% yield.

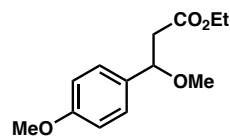
In the appropriate reaction vessel, aldol adduct (3–4 mmol, 1.0 equiv.), Ag₂O (9–10 mmol, 2.5–3.0 equiv.), and solvent (10–15 mL) were combined, followed by iodomethane (40–60 mmol, 10–15 equiv., **General Procedure B-1** or 9 mmol, 3.0 equiv., **General Procedure B-2**). The reaction was then stirred at the specified temperature for 24–48 hours, with additional equivalents of iodomethane (10–20 mmol, 2.5–5 equiv., **General Procedure B-1**) added as necessary. For **General Procedure B-1**, the reaction was performed in a vial equipped with a pressure release cap, toluene was employed as the solvent, and the reaction mixture was stirred at 70 °C. For **General Procedure B-2**, diethyl ether was employed as solvent and the reaction mixture was stirred at room temperature; the reaction vessel was covered in aluminum foil to protect its contents from light. The crude mixture was filtered through a pad of Celite and concentrated under reduced pressure. Purification was performed by silica column chromatography with hexanes / ethyl acetate; if necessary, a second purification by reverse phase chromatography was performed (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / acetonitrile eluent system). The desired products were obtained in 25–57% yield.

General Procedure C: Horner-Wadsworth-Emmons reaction and Pd/C alkene reduction synthetic sequence

In a dry round bottom flask, under argon, NaH (60% dispersion in mineral oil, 7.4–12 mmol, 1.1–2.0 equiv.) in anhydrous THF (8–23 mL) was cooled to 0 °C. Triethyl phosphonoacetate (7.4–18 mmol, 1.1–3.0 equiv.) was added dropwise and the mixture was allowed to warm to room temperature and stirred for 1 hour. Ketone (5–6.7 mmol, 1.0 equiv.) in THF (2–4 mL) was added and the reaction was stirred at room temperature for 12–18 hours (for the preparation of **3j** and **3l**) or heated to reflux (for the preparation of **3i**, **3k**, **8a'**, and **8b'**). The reaction was quenched with NH₄Cl (sat. aq., 20 mL). Phases were separated and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). The combined organics were washed with brine (10–20 mL), dried over Na₂SO₄, and concentrated under reduced pressure. When necessary, the crude product was purified by silica column chromatography with hexanes / ethyl acetate to afford the desired alkene compounds in 23% to quantitative yield.

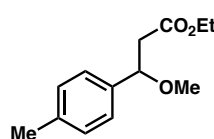
To a round bottom flask were added Pd/C (10% Pd on activated charcoal, 24–30% w/w of alkene), methanol (5–6 mL), and alkene (1.2–2.3 mmol). H₂ was bubbled through the solution for ~30 minutes. The reaction was stirred at room temperature under 1 atm H₂ until complete reduction of the alkene was observed by TLC (typically 3–8 hours). The crude product was filtered through a pad of Celite and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded the desired products in quantitative yield.

Ethyl 3-methoxy-3-(4-methoxyphenyl)propanoate (**3a**)



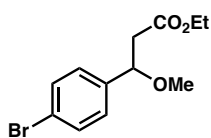
This compound was prepared from 4-methoxybenzaldehyde using **General Procedure B-1**. ¹H NMR (400 MHz, CDCl₃) δ 7.25 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 4.58 (dd, *J* = 9.0, 4.9 Hz, 1H), 4.14 (qd, *J* = 7.1, 1.2 Hz, 2H), 3.81 (s, 3H), 3.19 (s, 3H), 2.80 (dd, *J* = 15.2, 9.0 Hz, 1H), 2.55 (dd, *J* = 15.2, 4.9 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

Ethyl 3-methoxy-3-(*p*-tolyl)propanoate (**3b**)



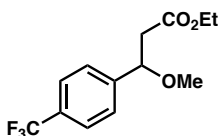
This compound was prepared from 4-methylbenzaldehyde using **General Procedure B-1**. ¹H NMR (400 MHz, CDCl₃) δ 7.22 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 7.9 Hz, 2H), 4.60 (dd, *J* = 9.2, 4.7 Hz, 1H), 4.14 (qd, *J* = 7.1, 1.2 Hz, 2H), 3.21 (s, 3H), 2.79 (dd, *J* = 15.3, 9.2 Hz, 1H), 2.55 (dd, *J* = 15.3, 4.7 Hz, 1H), 2.35 (s, 3H), 1.24 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.2, 137.9, 137.6, 129.4, 126.7, 80.0, 60.7, 56.9, 43.7, 21.3, 14.3. HRMS (FAB) *m/z*: 221.1169 [(*M* + H⁺) – H₂]; calc. for C₁₃H₁₇O₃: 221.1178

Ethyl 3-(4-bromophenyl)-3-methoxypropanoate (3c)



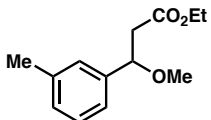
This compound was prepared from 4-bromobenzaldehyde using **General Procedure B-1**. ^1H NMR (400 MHz, CDCl_3) δ 7.48 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.3 Hz, 2H), 4.59 (dd, J = 8.9, 5.0 Hz, 1H), 4.14 (qd, J = 7.1, 0.7 Hz, 2H), 3.21 (s, 3H), 2.77 (dd, J = 15.4, 8.9 Hz, 1H), 2.53 (dd, J = 15.4, 5.0 Hz, 1H), 1.23 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.8, 139.8, 131.9, 128.5, 122.0, 79.6, 60.8, 57.1, 43.5, 14.3. **HRMS** (FAB) m/z : 287.0282 ($\text{M} + \text{H}^+$); calc. for $\text{C}_{12}\text{H}_{16}^{79}\text{BrO}_3$: 287.0283

Ethyl 3-methoxy-3-(4-(trifluoromethyl)phenyl)propanoate (3d)



This compound was prepared from 4-(trifluoromethyl)benzaldehyde using **General Procedure B-1**. ^1H NMR (400 MHz, CDCl_3) δ 7.62 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 4.70 (dd, J = 8.8, 4.9 Hz, 1H), 4.15 (qd, J = 7.2, 0.7 Hz, 2H), 3.24 (s, 3H), 2.79 (dd, J = 15.5, 8.9 Hz, 1H), 2.56 (dd, J = 15.4, 4.9 Hz, 1H), 1.24 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.7, 145.0, 130.4 (q, J = 32.4 Hz), 127.1, 125.7 (q, J = 3.8 Hz), 124.2 (q, J = 272.1 Hz), 79.7, 60.9, 57.3, 43.5, 14.3. **HRMS** (FAB) m/z : 277.1041 ($\text{M} + \text{H}^+$); calc. for $\text{C}_{13}\text{H}_{16}\text{F}_3\text{O}_3$: 277.1052

Ethyl 3-methoxy-3-(*m*-tolyl)propanoate (3e)



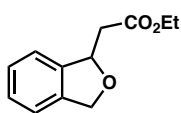
This compound was prepared from 3-methylbenzaldehyde using **General Procedure B-1**. ^1H NMR (400 MHz, CDCl_3) δ 7.27 – 7.21 (m, 1H), 7.16 – 7.08 (m, 3H), 4.60 (dd, J = 9.2, 4.6 Hz, 1H), 4.15 (qd, J = 7.1, 1.3 Hz, 2H), 3.22 (s, 3H), 2.79 (dd, J = 15.3, 9.3 Hz, 1H), 2.56 (dd, J = 15.3, 4.6 Hz, 1H), 2.36 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 171.2, 140.7, 138.3, 128.9, 128.6, 127.4, 123.8, 80.2, 60.7, 57.0, 43.7, 21.6, 14.3. **HRMS** (FAB) m/z : 223.1338 ($\text{M} + \text{H}^+$); calc. for $\text{C}_{13}\text{H}_{19}\text{O}_3$: 223.1334.

Ethyl 2-(1,3-dihydroisobenzofuran-1-yl)acetate (3f)

This compound was prepared by the method of U. S. Dakarapu *et al.*³³. To a flame-dried Schlenk flask under argon was added $[\text{Ir}(\text{coe})_2\text{Cl}]_2$ (5 mg, 0.0056 mmol, 0.11 mol%), phthalide (671 mg, 5 mmol, 1.0 equiv.), anhydrous dichloromethane (1.6 mL), and H_2SiEt_2 (1.3 mL, 10 mmol, 2 equiv.). The reaction mixture was stirred for 14 hours at room temperature. The reaction mixture was concentrated under reduced pressure to afford the crude silyl acetal, which was used without purification.

In a dry round bottom flask, the crude silyl acetal (5 mmol, 1.0 equiv.) was combined with THF (5 mL) and the resulting mixture cooled to 0 °C. To the mixture were added triethyl phosphonoacetate (1.23 g, 5.5 mmol, 1.1 equiv.) and KOSiMe_3 (713 mg, 5 mmol, 1.0 equiv.) in THF (7.5 mL). The reaction was allowed to warm to room temperature and stirred for 1.5 hours. The reaction was quenched with the addition of NH_4Cl (sat. aq., 12 mL) and the aqueous phase was extracted with diethyl ether (3×15 mL). The combined organics were washed with brine (15 mL), dried over Na_2SO_4 , and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded desired product **3f** with impurities (667 mg,

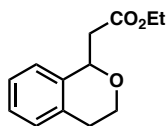
3.2 mmol, 65% yield). A portion of the product was taken for a second purification by reverse phase chromatography (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / acetonitrile eluent system).



Spectral data are in agreement with literature report³³. ¹H NMR (400 MHz, CDCl₃) δ 7.33 – 7.26 (m, 2H), 7.25 – 7.16 (m, 2H), 5.71 – 5.63 (m, 1H), 5.19 – 5.13 (m, 1H), 5.11 – 5.04 (m, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 2.80 (dd, *J* = 15.6, 4.9 Hz, 1H), 2.73 (dd, *J* = 15.6, 7.9 Hz, 1H), 1.27 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.0, 140.8, 139.3, 128.0, 127.6, 121.3, 121.2, 80.5, 72.9, 60.8, 41.8, 14.3.

Ethyl 2-(isochroman-1-yl)acetate (3g)

This compound was prepared by the method of R. E. TenBrink *et al.*³⁴. To a 100 mL dry round bottom flask, under argon, were added 2-phenylethanol (1.47 g, 12 mmol, 1.0 equiv.), ethyl 3,3-diethoxypropionate (90% technical grade, 2.51 g, 13.2 mmol, 1.1 equiv.), and anhydrous dichloromethane (5 mL). The resulting mixture was cooled to 0 °C and TiCl₄ (1 M in dichloromethane, 26.4 mL, 26.4 mmol, 2.2 equiv.) was added slowly. The reaction was stirred for 2 hours at 0 °C and a second portion of ethyl 3,3-diethoxypropionate (90% technical grade, 0.12 g, 0.6 mmol, 0.05 equiv.) was added. The reaction was stirred for an additional 2 hours at 0 °C. The mixture was poured into ice cold HCl (aq., 1 M, 20 mL) and the aqueous phase was extracted with dichloromethane (2 x 20 mL). The combined organics were washed with brine (30 mL), dried over Na₂SO₄, and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded desired product **3g** with minor impurities (2.59 g, ~11.8 mmol, ~98% yield). A portion of the product was taken for a second purification by reverse phase chromatography (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / acetonitrile eluent system).

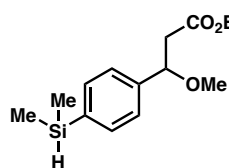


Spectral data are in agreement with literature report³⁴. ¹H NMR (400 MHz, CDCl₃) δ 7.22 – 7.15 (m, 2H), 7.15 – 7.09 (m, 1H), 7.08 – 7.02 (m, 1H), 5.25 (dd, *J* = 9.6, 3.5 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 4.13 (ddd, *J* = 11.4, 5.2, 4.2 Hz, 1H), 3.82 (ddd, *J* = 11.4, 9.0, 3.9 Hz, 1H), 3.04 – 2.93 (m, 1H), 2.88 (dd, *J* = 15.2, 3.6 Hz, 1H), 2.80 – 2.68 (m, 2H), 1.28 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.4, 136.9, 134.1, 129.2, 126.8, 126.4, 124.6, 73.1, 63.2, 60.8, 41.9, 28.9, 14.3.

Ethyl 3-(4-(dimethylsilyl)phenyl)-3-methoxypropanoate (3h)

This compound was prepared from ethyl 3-(4-bromophenyl)-3-methoxypropanoate (**3c**). The following procedure was modified from the literature³⁵. To a 25 mL round bottom flask was added Mg turnings* (48 mg, 2.0 mmol, 2.0 equiv.), flame dried, and cooled under positive argon pressure. (*Mg turnings were prepared by washing with 0.1 M HCl, sonication, then washing with H₂O and acetone.) THF (3 mL), LiCl (64 mg, 1.5 mmol, 1.5 equiv.), and Me₂SiHCl (170 mg, 1.8 mmol, 1.8 equiv.) were added and the resulting mixture was stirred for 30 minutes at room temperature under positive argon pressure. Aryl bromide **3c** (287 mg, 1.0 mmol, 1.0 equiv.) was added dropwise via syringe and the reaction was stirred for an additional 2 hours. The crude reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded desired

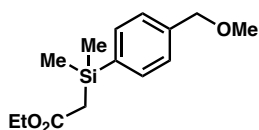
product **3h** (145 mg, 0.54 mmol, 54% yield).



¹H NMR (400 MHz, CDCl₃) δ 7.54 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.9 Hz, 2H), 4.63 (dd, *J* = 9.3, 4.5 Hz, 1H), 4.42 (hept, *J* = 3.8 Hz, 1H), 4.15 (qd, *J* = 7.1, 1.6 Hz, 2H), 3.23 (s, 3H), 2.79 (dd, *J* = 15.3, 9.3 Hz, 1H), 2.56 (dd, *J* = 15.3, 4.5 Hz, 1H), 1.24 (t, *J* = 7.2 Hz, 3H), 0.35 (d, *J* = 3.8 Hz, 6H). **¹³C NMR** (101 MHz, CDCl₃) δ 171.1, 141.8, 137.4, 134.4, 126.2, 80.2, 60.7, 57.1, 43.7, 14.3, -3.6. **HRMS** (FAB) *m/z*: 265.1253 [(*M* + *H*⁺) - H₂]; calc. for C₁₄H₂₁SiO₃: 265.1260.

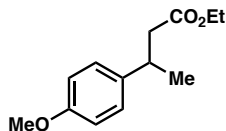
Ethyl 2-((4-(methoxymethyl)phenyl)dimethylsilyl)acetate (**3h'**)

This compound was prepared by rhodium-catalyzed Si-H insertion. To a dry 50 mL round bottom flask, under argon, was added (4-(methoxymethyl)phenyl)dimethylsilane (**1h**) (541 mg, 3 mmol, 1.0 equiv.), Rh₂(OAc)₄ (13.3 mg, ~1 mol%), and anhydrous dichloromethane (12 mL). The mixture was cooled to -78 °C, after which ethyl diazoacetate (393 mg, 3.0 mmol, 1.0 equiv.) in dichloromethane (3 mL) was added dropwise to the solution over 2 hours. The reaction was allowed to slowly warm to room temperature and stirred for a total of 12 hours. The crude reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The crude mixture was purified by silica column chromatography using hexanes / ethyl acetate to deliver **3h'** with impurities. A second purification by silica column chromatography using hexanes / diethyl ether/ dichloromethane afforded **3h'** (92.6 mg, 0.35 mmol, 12% yield).



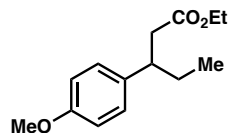
¹H NMR (400 MHz, CDCl₃) δ 7.52 (d, *J* = 8.1 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 4.46 (s, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 3.39 (s, 3H), 2.11 (s, 2H), 1.16 (t, *J* = 7.1 Hz, 3H), 0.40 (s, 6H). **¹³C NMR** (101 MHz, CDCl₃) δ 172.7, 139.7, 136.4, 133.8, 127.2, 74.6, 60.1, 58.3, 26.4, 14.5, -2.6. **HRMS** (FAB) *m/z*: 265.1260 [(*M* + *H*⁺) - H₂]; calc. for C₁₄H₂₁SiO₃: 265.1260.

Ethyl 3-(4-methoxyphenyl)butanoate (**3i**)



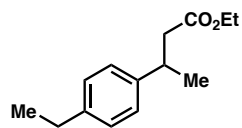
This compound was prepared from 1-(4-methoxyphenyl)ethan-1-one using **General Procedure C**. **¹H NMR** (500 MHz, CDCl₃) δ 7.14 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.08 (qd, *J* = 7.2, 1.2 Hz, 2H), 3.79 (s, 3H), 3.24 (h, *J* = 7.1 Hz, 1H), 2.57 (dd, *J* = 14.9, 7.2 Hz, 1H), 2.51 (dd, *J* = 14.9, 8.0 Hz, 1H), 1.28 (d, *J* = 7.0 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

Ethyl 3-(4-methoxyphenyl)pentanoate (**3j**)



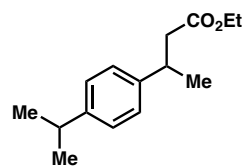
This compound was prepared from 1-(4-methoxyphenyl)propan-1-one using **General Procedure C**. Spectral data are in agreement with literature report³⁶. **¹H NMR** (400 MHz, CDCl₃) δ 7.09 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 4.03 (qd, *J* = 7.2, 1.3 Hz, 2H), 3.78 (s, 3H), 2.95 (tdd, *J* = 9.0, 7.0, 5.3 Hz, 1H), 2.60 (dd, *J* = 15.0, 7.0 Hz, 1H), 2.51 (dd, *J* = 14.9, 8.3 Hz, 1H), 1.68 (ddq, *J* = 13.3, 7.4, 5.4 Hz, 1H), 1.56 (ddq, *J* = 13.5, 9.4, 7.3 Hz, 1H), 1.14 (t, *J* = 7.1 Hz, 3H), 0.78 (t, *J* = 7.4 Hz, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 172.7, 158.2, 136.1, 128.5, 113.8, 60.3, 55.3, 43.3, 41.9, 29.4, 14.3, 12.1.

Ethyl 3-(4-ethylphenyl)butanoate (**3k**)



This compound was prepared from 1-(4-ethylphenyl)ethan-1-one using **General Procedure C**. ^1H NMR (400 MHz, CDCl_3) δ 7.14 (*app.* s, 4H), 4.08 (q, J = 7.1 Hz, 2H), 3.25 (dp, J = 8.3, 7.0 Hz, 1H), 2.66 – 2.48 (m, 4H), 1.29 (d, J = 6.9 Hz, 3H), 1.26 – 1.15 (m, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 172.7, 143.1, 142.3, 128.0, 126.8, 60.4, 43.2, 36.2, 28.5, 22.0, 15.7, 14.3. **HRMS** (FAB) m/z : 221.1532 ($\text{M} + \text{H}^+$); calc. for $\text{C}_{14}\text{H}_{21}\text{O}_2$: 221.1542

Ethyl 3-(4-isopropylphenyl)butanoate (**3l**)

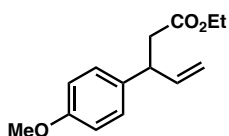


This compound was prepared from 1-(4-isopropylphenyl)ethan-1-one using **General Procedure C**. ^1H NMR (400 MHz, CDCl_3) δ 7.15 (*app.* s, 4H), 4.08 (q, J = 7.1 Hz, 2H), 3.25 (dp, J = 8.5, 6.9 Hz, 1H), 2.87 (hept, J = 6.9 Hz, 1H), 2.60 (dd, J = 14.9, 6.7 Hz, 1H), 2.51 (dd, J = 14.9, 8.4 Hz, 1H), 1.29 (d, J = 7.0 Hz, 3H), 1.23 (d, J = 6.9 Hz, 6H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 172.7, 147.0, 143.2, 126.8, 126.6, 60.4, 43.3, 36.2, 33.8, 24.2, 21.9, 14.3. **HRMS** (FAB) m/z : 235.1696 ($\text{M} + \text{H}^+$); calc. for $\text{C}_{15}\text{H}_{23}\text{O}_2$: 235.1698.

Ethyl 3-(4-methoxyphenyl)pent-4-enoate (**3m**)

This compound was accessed in a two-step sequence. First, *p*-methoxycinnamaldehyde (811 mg, 5 mmol, 1.0 equiv.) was reduced using NaBH_4 (227 mg, 6 mmol, 1.2 equiv.) in methanol (15 mL) under standard reaction conditions (0 °C for 2 hours). The reaction mixture was quenched with NH_4Cl (sat. aq., 10 mL) and diluted with dichloromethane (15 mL). Phases were separated and the aqueous layer was extracted with dichloromethane (4×15 mL). The combined organics were washed with brine (25 mL), dried over Na_2SO_4 , and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate delivered *p*-methoxycinnamyl alcohol (752 mg, 4.6 mmol, 92% yield), with spectral data that match literature report³⁷.

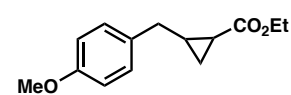
Next, to a 50 mL round bottom flask equipped with short-path condenser were added *p*-methoxycinnamyl alcohol (740 mg, 4.5 mmol, 1.0 equiv.), triethyl orthoacetate (7.3 g, 45 mmol, 10 equiv.), and propionic acid (52 mg, 0.7 mmol, 0.15 equiv.). Following standard Johnson-Claisen rearrangement conditions, this mixture was heated to 140 °C until complete conversion of *p*-methoxycinnamyl alcohol was observed by TLC (~23 hours). Additional propionic acid (2×52 mg) was added after 6 hours and 9 hours reaction time. The reaction mixture was removed from heat, concentrated under reduced pressure, and purified using silica gel chromatography with hexanes / ethyl acetate as eluents. A second purification by silica gel chromatography with hexanes / ether afforded **3m** (357 mg, 1.6 mmol, 36% yield).



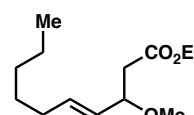
Spectral data for **3m** are in agreement with literature report³⁸. ^1H NMR (400 MHz, CDCl_3) δ 7.13 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 5.96 (ddd, J = 17.5, 9.9, 6.9 Hz, 1H), 5.09 – 5.05 (m, 1H), 5.03 (dt, J = 5.4, 1.3 Hz, 1H), 4.07 (qd, J = 7.1, 1.0 Hz, 2H), 3.86 – 3.80 (m, 1H), 3.78 (s, 3H), 2.73 (dd, J = 15.0, 8.0 Hz, 1H), 2.65 (dd, J = 15.0, 7.6 Hz, 1H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 172.1, 158.4, 140.7, 134.6, 128.6, 114.6, 114.0, 60.5, 55.4, 44.9, 40.6, 14.3.

Ethyl 2-(4-methoxybenzyl)cyclopropane-1-carboxylate (**3m'**)

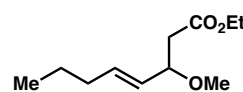
This compound was prepared by rhodium-catalyzed alkene cyclopropanation. To a dry 100 mL round bottom flask, under argon, were added 4-allylanisole (3.0 g, 20 mmol, 10 equiv.), Rh₂(OAc)₄ (8.8 mg, ~1 mol%), and anhydrous dichloromethane (10 mL). Ethyl diazoacetate (262 mg, 2 mmol, 1.0 equiv.) in dichloromethane (10 mL) was added over ~8 hours using a syringe pump; the reaction mixture was allowed to stir for a total of 20 hours at room temperature. The reaction mixture was diluted with diethyl ether (20 mL), filtered through a pad of Celite, and concentrated under reduced pressure. Several rounds of purification by silica column chromatography with hexanes / ethyl acetate or hexanes / diethyl ether eluent systems afforded *cis*-**3m'** and *trans*-**3m'** as individual isomers (combined mass 148.1 mg, 0.632 mmol, 32% yield).

 Spectral data are in agreement with literature report³⁹. Characterization data for *cis*-**3m'**: ¹H NMR (400 MHz, CDCl₃) δ 7.13 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 2.86 (dd, *J* = 14.9, 6.9 Hz, 1H), 2.77 (dd, *J* = 15.0, 7.6 Hz, 1H), 1.77 (ddd, *J* = 8.8, 7.6, 5.9 Hz, 1H), 1.56 – 1.44 (m, 1H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.14 – 1.06 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 173.1, 158.0, 133.7, 129.3, 113.9, 60.5, 55.4, 32.1, 23.1, 18.7, 14.5, 13.7. Characterization data for *trans*-**3m'**: ¹H NMR (400 MHz, CDCl₃) δ 7.12 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.11 (qd, *J* = 7.1, 1.1 Hz, 2H), 3.79 (s, 3H), 2.71 (dd, *J* = 14.7, 6.3 Hz, 1H), 2.52 (dd, *J* = 14.8, 7.1 Hz, 1H), 1.65 (ddtd, *J* = 8.7, 7.1, 6.4, 4.1 Hz, 1H), 1.52 – 1.46 (m, 1H), 1.27 – 1.20 (m, 4H), 0.81 (ddd, *J* = 8.2, 6.3, 4.2 Hz, 1H). ¹³C NMR (101 MHz, CDCl₃) δ 174.4, 158.2, 132.3, 129.5, 113.9, 60.5, 55.4, 37.6, 23.4, 20.3, 15.3, 14.4.

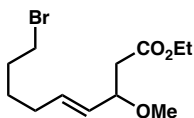
Ethyl (*E*)-3-methoxydec-4-enoate (**5a**)

 This compound was prepared from (*E*)-oct-2-enal using **General Procedure B-2**. ¹H NMR (400 MHz, CDCl₃) δ 5.69 (dt, *J* = 15.4, 6.8 Hz, 1H), 5.28 (ddt, *J* = 15.4, 8.3, 1.5 Hz, 1H), 4.14 (qd, *J* = 7.2, 0.8 Hz, 2H), 3.97 (td, *J* = 8.2, 5.5 Hz, 1H), 3.25 (s, 3H), 2.59 (dd, *J* = 14.9, 8.1 Hz, 1H), 2.42 (dd, *J* = 14.9, 5.5 Hz, 1H), 2.10 – 1.97 (m, 2H), 1.43 – 1.20 (m, 9H), 0.88 (t, *J* = 6.9 Hz, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

Ethyl (*E*)-3-methoxyoct-4-enoate (**5b**)

 This compound was prepared from (*E*)-hex-2-enal using **General Procedure B-2**. ¹H NMR (400 MHz, CDCl₃) δ 5.69 (dt, *J* = 15.4, 6.8 Hz, 1H), 5.29 (ddt, *J* = 15.4, 8.2, 1.5 Hz, 1H), 4.14 (qd, *J* = 7.1, 0.8 Hz, 2H), 3.97 (td, *J* = 8.1, 5.5 Hz, 1H), 3.25 (s, 3H), 2.59 (dd, *J* = 14.9, 8.1 Hz, 1H), 2.42 (dd, *J* = 14.9, 5.6 Hz, 1H), 2.06 – 1.99 (m, 2H), 1.40 (sext, *J* = 7.3 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.2, 135.3, 128.9, 79.0, 60.6, 56.2, 41.5, 34.3, 22.4, 14.4, 13.7. HRMS (FAB) *m/z*: 199.1320 [(*M* + *H*⁺) – H₂]; calc. for C₁₁H₁₉O₃: 199.1334.

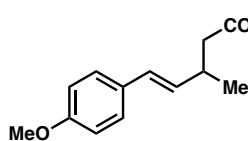
Ethyl (*E*)-9-bromo-3-methoxynon-4-enoate (**5c**)



This compound was prepared from (*E*)-7-bromohept-2-enal using **General Procedure B-2**. The synthesis of (*E*)-7-bromohept-2-enal was described in the synthesis of compound **4c** in **Section VI**. $^1\text{H NMR}$ (400 MHz, Chloroform-*d*) δ 5.67 (dt, $J = 15.4, 6.7$ Hz, 1H), 5.31 (dd, $J = 15.4, 8.1$ Hz, 1H), 4.13 (q, $J = 7.1$ Hz, 2H), 3.97 (td, $J = 8.0, 5.5$ Hz, 1H), 3.40 (t, $J = 6.7$ Hz, 2H), 3.25 (s, 3H), 2.59 (dd, $J = 15.0, 8.0$ Hz, 1H), 2.41 (dd, $J = 15.0, 5.6$ Hz, 1H), 2.08 (q, $J = 7.2$ Hz, 2H), 1.91 – 1.79 (m, 2H), 1.53 (p, $J = 7.5$ Hz, 2H), 1.25 (t, $J = 7.2$ Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 171.1, 134.3, 129.5, 78.8, 60.6, 56.3, 41.4, 33.7, 32.2, 31.4, 27.7, 14.4. **HRMS** (FAB) m/z : 293.0764 ($\text{M} + \text{H}^+$); calc. for $\text{C}_{12}\text{H}_{22}\text{O}_3^{79}\text{Br}$: 293.0752.

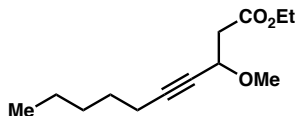
Ethyl (*E*)-5-(4-methoxyphenyl)-3-methylpent-4-enoate (**5d**)

To a 6 mL vial equipped with a stir bar was added Grubbs' catalyst 2nd generation (10 mg, 2 mol%). The vial was then evacuated and backfilled with argon for three times. Under argon, a dry CH_2Cl_2 solution (2 mL) containing 4-vinylanisole (100 mg, 0.75 mmol) and ethyl 3-methylpent-4-enoate (503 mg, 3.75 mmol) was added to the vial via syringe. The mixture was stirred at 40 °C for 24 hours and then cooled to room temperature and filtered through a silica plug. The solvent was removed under reduced pressure and the crude product was purified using silica column chromatography with hexanes / ethyl acetate to give **5d** (37 mg, 20% yield).



$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.30 – 7.24 (m, 2H), 6.84 (d, $J = 8.8$ Hz, 2H), 6.34 (d, $J = 15.9$ Hz, 1H), 5.99 (dd, $J = 15.9, 7.6$ Hz, 1H), 4.12 (q, $J = 7.1$ Hz, 2H), 3.80 (s, 3H), 2.90 – 2.75 (m, 1H), 2.41 (dd, $J = 14.7, 7.3$ Hz, 1H), 2.34 (dd, $J = 14.7, 7.3$ Hz, 1H), 1.23 (t, $J = 7.1$ Hz, 3H), 1.14 (d, $J = 6.7$ Hz, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

Ethyl 3-methoxydec-4-ynoate (**5e**)

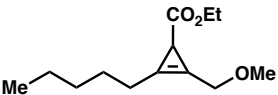


This compound was prepared from oct-2-ynal using **General Procedure B-2**. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.39 (ddt, $J = 8.3, 5.4, 2.0$ Hz, 1H), 4.16 (qd, $J = 7.2, 1.0$ Hz, 2H), 3.39 (s, 3H), 2.73 (dd, $J = 15.5, 8.4$ Hz, 1H), 2.63 (dd, $J = 15.5, 5.4$ Hz, 1H), 2.20 (td, $J = 7.1, 2.0$ Hz, 2H), 1.50 (p, $J = 7.2$ Hz, 2H), 1.41 – 1.29 (m, 4H), 1.26 (t, $J = 7.1$ Hz, 3H), 0.89 (t, $J = 7.1$ Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 170.4, 87.3, 67.7, 60.8, 56.6, 41.7, 31.1, 28.4, 22.3, 18.8, 14.3, 14.1 (one carbon may be overlapping with the solvent peaks). **HRMS** (FAB) m/z : 227.1638 ($\text{M} + \text{H}^+$); calc. for $\text{C}_{13}\text{H}_{23}\text{O}_3$: 227.1647.

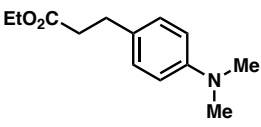
Ethyl 2-(methoxymethyl)-3-pentylcycloprop-2-ene-1-carboxylate (**5e'**)

This compound was prepared by rhodium-catalyzed cyclopropanation. To a dry 50 mL round bottom flask was added 1-methoxyoct-2-yne (**4e**) (280 mg, 2.0 mmol, 1.0 equiv.), $\text{Rh}_2(\text{OAc})_4$ (9.0 mg, 1 mol%), and anhydrous dichloromethane (6 mL). The mixture was cooled to -78 °C, after which ethyl diazoacetate (87%, 525 mg, 4.0 mmol, 2.0 equiv.) in dichloromethane (5 mL) was added dropwise to the solution over 6 hours. The reaction was allowed to slowly warm to room temperature and stirred for a total of 18 hours. The reaction mixture was concentrated

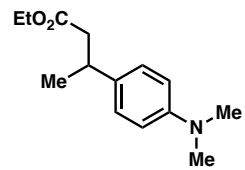
under reduced pressure. The crude product was purified by silica column chromatography using hexanes / ethyl acetate, followed by C18 column using methanol / water, to afford **5e'** (26 mg, 0.11 mmol, 6% yield).

 **¹H NMR** (400 MHz, CDCl₃) δ 4.37 (t, *J* = 1.6 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.39 (s, 3H), 2.47 (tt, *J* = 7.5, 1.6 Hz, 2H), 2.20 (s, 1H), 1.64 – 1.52 (m, 2H), 1.37 – 1.28 (m, 4H), 1.24 (t, *J* = 7.1 Hz, 3H), 0.93 – 0.86 (m, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 176.2, 110.3, 102.5, 65.8, 60.2, 58.6, 31.5, 26.7, 24.7, 22.7, 22.5, 14.5, 14.1. **HRMS** (EI) *m/z*: 226.1573 (*M*⁺); calc. for C₁₃H₂₂O₃: 226.1569.

Ethyl 3-(4-(dimethylamino)phenyl)propanoate (**8a'**)

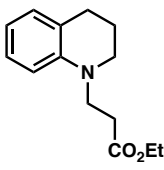
 This compound was prepared from 4-(dimethylamino)benzaldehyde using **General Procedure C**. **¹H NMR** (400 MHz, CDCl₃) δ 7.09 (d, *J* = 8.7 Hz, 2H), 6.70 (d, *J* = 8.7 Hz, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 2.92 (s, 6H), 2.89 – 2.82 (m, 2H), 2.61 – 2.54 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 173.4, 149.4, 129.0, 128.8, 113.1, 60.4, 41.0, 36.6, 30.2, 14.4. **HRMS** (EI) *m/z*: 221.1430 (*M*⁺); calc. for C₁₃H₁₉NO₂: 221.1416.

Ethyl 3-(4-(dimethylamino)phenyl)butanoate (**8b'**)

 This compound was prepared from 1-(4-(dimethylamino)phenyl)ethan-1-one using **General Procedure C**. Spectral data are in agreement with literature report⁴⁰. **¹H NMR** (400 MHz, CDCl₃) δ 7.10 (d, *J* = 8.7 Hz, 2H), 6.70 (d, *J* = 8.7 Hz, 2H), 4.08 (qd, *J* = 7.1, 1.1 Hz, 2H), 3.20 (dt, *J* = 8.4, 6.8 Hz, 1H), 2.92 (s, 6H), 2.57 (dd, *J* = 14.8, 6.8 Hz, 1H), 2.49 (dd, *J* = 14.8, 8.4 Hz, 1H), 1.27 (d, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 172.8, 149.4, 134.0, 127.4, 113.0, 60.3, 43.5, 41.0, 35.7, 22.0, 14.4.

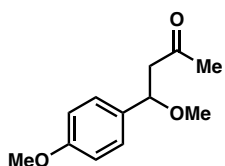
Ethyl 3-(3,4-dihydroquinolin-1(2*H*)-yl)propanoate (**8f'**)

To a 100-mL round-bottom flask were added 1,2,3,4-tetrahydroquinoline (266.4 mg, 2.0 mmol, 1.0 equiv.), ethyl 3-bromopropanoate (0.97 mL, 6.0 mmol, 3.0 equiv.), K₂CO₃ (0.552 g, 4.0 mmol, 2.0 equiv.), KI (66.0 mg, 0.4 mmol, 0.2 equiv.) and *N,N*-dimethylformamide (30 mL). The reaction mixture was heated at 120 °C for 4 hours. After the reaction was cooled to room temperature and quenched by H₂O (40 mL), the crude product was extracted by diethyl ether (20 mL × 3). The combined organic layer was washed by H₂O (40 mL) and brine (40 mL), and then dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with pentane / diethyl ether, followed by C18 column with methanol / water, to afford **8f'** (350 mg, 1.5 mmol, 75% yield).

 This compound is known in the literature⁴¹. **¹H NMR** (400 MHz, CDCl₃) δ 7.11 – 7.01 (m, 1H), 6.95 (dq, *J* = 7.1, 1.1 Hz, 1H), 6.66 – 6.54 (m, 2H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.65 – 3.57 (m, 2H), 3.33 – 3.25 (m, 2H), 2.75 (t, *J* = 6.4 Hz, 2H), 2.64 – 2.54 (m, 2H), 1.99 – 1.89 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 172.6, 144.6, 129.5, 127.3, 122.8, 116.2, 110.7, 60.7, 49.5, 47.4, 31.5, 28.1, 22.3, 14.4.

4-Methoxy-4-(4-methoxyphenyl)butan-2-one (10d)

This compound was prepared according to the procedure of Yadav *et al*⁴². Briefly, a mixture of 4-anisaldehyde (10 mmol), 2,2-dimethoxypropane (20 mmol) and iodine (0.2 mmol) in dry methylene chloride (20 mmol) was stirred under N₂ for 30 min. After the reaction was complete as indicated by TLC, the reaction mixture was diluted with water and extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with sodium thiosulfate (aq., 15% w/v) and brine, and then dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica column chromatography with hexanes / ethyl acetate.



¹H NMR (300 MHz, CDCl₃) δ 7.25 – 7.21 (m, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 4.58 (dd, *J* = 8.8, 4.5 Hz, 1H), 3.79 (s, 3H), 3.16 (s, 3H), 3.05 – 2.88 (m, 1H), 2.57 (dd, *J* = 15.8, 4.5 Hz, 1H), 2.14 (s, 3H). Spectral data are in agreement with that for the enzymatic product (see **Section IX**).

VIII. Small scale enzymatic reactions and product calibration curves

Enzymatic reactions performed on analytical scale were conducted following the general procedure described below, also described in **Section I (H)** and **Section I (I)**. Product formation was quantified by HPLC or GC based on the calibration curve of the corresponding racemic reference compound (**Section VII**). TTN is defined as the amount of product divided by total heme protein as measured by the hemochrome assay (**Section I (G)**). Analysis data presented in this section are for results shown in Fig. 3 and Fig. 4 of the main text.

General procedure for biotransformations using whole *E. coli* cells

Suspensions of *E. coli* expressing the appropriate heme protein variant in M9-N buffer ($OD_{600} = 30$, for data in **Section VIII**) were degassed by bubbling with argon in sealed vials for at least 40 minutes; the cells were kept on ice during this time. Separately, a solution of *D*-glucose (250 mM in M9-N) was degassed by sparging with argon for at least 30 minutes. All solutions were then transferred into an anaerobic chamber for reaction set up. To a 2 mL vial were added a GOX oxygen depletion solution (20 μ L of stock solution containing 14,000 U/mL catalase and 1,000 U/mL glucose oxidase in 0.1 M potassium phosphate buffer, pH 8.0), *D*-glucose (40 μ L of 250 mM stock solution in M9-N buffer), degassed suspension of *E. coli* expressing heme protein ($OD_{600} = 30$, 320 μ L), alkane substrate (10 μ L of 400 mM stock solution in EtOH), and ethyl diazoacetate (10 μ L of 400 mM stock solution in EtOH) in the listed order. Final reaction volume was 400 μ L; final concentrations were 10 mM alkane substrate, 10 mM ethyl diazoacetate, and 25 mM *D*-glucose. **Note:** reaction performed with *E. coli* cells resuspended to $OD_{600} = 30$ indicates that 320 μ L of $OD_{600} = 30$ cells were added, and likewise for other reaction OD_{600} descriptions. The vials were sealed, removed from the anaerobic chamber, and shaken at room temperature and 500 rpm for 18 hours. The expression of heme protein was measured using the hemochrome assay (**Section I (G)**), and the concentration of heme protein in the biotransformation was calculated accordingly.

Reaction workup for quantitative HPLC analysis. The reactions were quenched by addition of acetonitrile (400 μ L) and internal standard (10 μ L of 60 mM ethyl phenoxyacetate or 40 mM ethyl benzoate in acetonitrile, as appropriate). This mixture was then transferred to a microcentrifuge tube, vortexed (10 seconds, 3 times) and centrifuged ($20,000 \times g$, 10 minutes). The supernatant was taken for HPLC analysis.

Reaction workup for quantitative GC analysis. Internal standard 1,3,5-trimethoxybenzene (10 μ L of 40 mM stock solution in cyclohexane) was added to the reaction vial followed by mixed solvent (cyclohexane / ethyl acetate = 1 : 1, 800 μ L). The mixture was transferred to a 1.5 mL microcentrifuge tube, vortexed (10 seconds, 3 times), and centrifuged ($20,000 \times g$, 5 minutes) to completely separate the organic and aqueous layers. The organic layer was taken for GC analysis.

HPLC calibration curve preparation

Stock solutions of chemically synthesized products at various concentrations (1 to 200 mM in EtOH) were prepared. To a 2 mL vial were added 380 μ L water, 20 μ L product stock solution, 10 μ L internal standard (60 mM ethyl phenoxyacetate or 40 mM ethyl benzoate in acetonitrile, as appropriate) and 400 μ L acetonitrile. The mixture was vortexed and analyzed by HPLC. Data points represent the average of two runs. The standard curves plot product concentration in mM

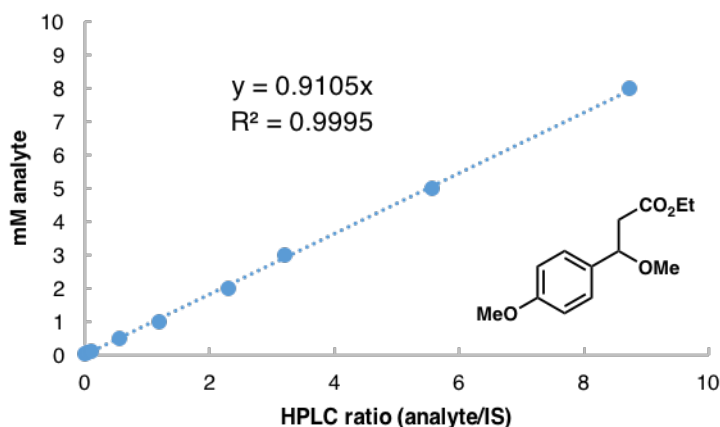
(y-axis) against the ratio of product area to internal standard area on the HPLC (x-axis).

GC calibration curve preparation

Stock solutions of chemically synthesized products at various concentrations (0.2 to 200 mM in EtOH) were prepared. To a microcentrifuge tube were added 380 μ L M9-N buffer, 20 μ L product stock solution, 10 μ L internal standard (40 mM 1,3,5-trimethoxybenzene in cyclohexane), and 800 μ L mixed solvent system (cyclohexane : ethyl acetate = 1:1). The mixture was vortexed (10 seconds, 3 times) then centrifuged ($20,000 \times g$, 5 min) to completely separate the organic and aqueous layers. The organic layer was removed for GC analysis. All data points represent the average of at least two runs. The standard curves plot product concentration in mM (y-axis) against the ratio of product area to internal standard area on the GC (x-axis).

Ethyl 3-methoxy-3-(4-methoxyphenyl)propanoate (3a)

HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm



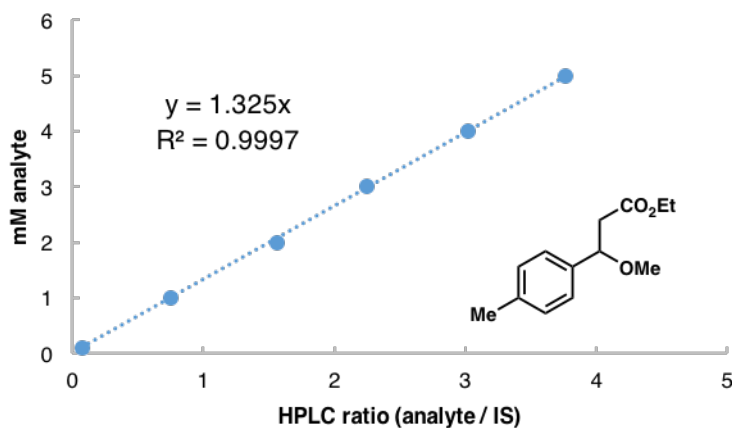
Analysis data for **P411-CHF** catalyzed C–H alkylation of **1a** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3a -(1)	5457.7	1066.1	5.119	4.66	2.19	2133		
3a -(2)	5469.8	1137.5	4.809	4.38	2.19	2004		
3a -(3)	5740.7	1129.0	5.085	4.63	2.07	2238		
3a -(4)	5684.8	1133.4	5.016	4.57	2.07	2208	2150	100

Notes: Pdt = product area, IS = internal standard area, [Pdt] = product concentration in reaction, [PC] = protein concentration in reaction, Avg. TTN = average total turnover number, SD TTN = standard deviation of TTN. These notes apply for the following tables.

Ethyl 3-methoxy-3-(*p*-tolyl)propanoate (3b)

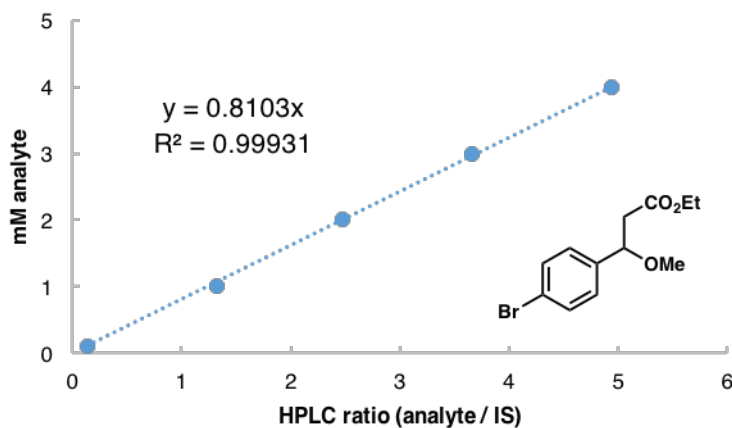
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm

Analysis data for **P411-CHF** catalyzed C–H alkylation of **1b** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3b -(1)	2719.0	2242.6	1.212	1.61	2.19	735		
3b -(2)	2760.9	2233.2	1.236	1.64	2.19	750		
3b -(3)	3257.6	2244.6	1.451	1.92	2.07	930		
3b -(4)	3275.5	2253.5	1.454	1.93	2.07	931	840	110

Ethyl 3-(4-bromophenyl)-3-methoxypropanoate (3c)

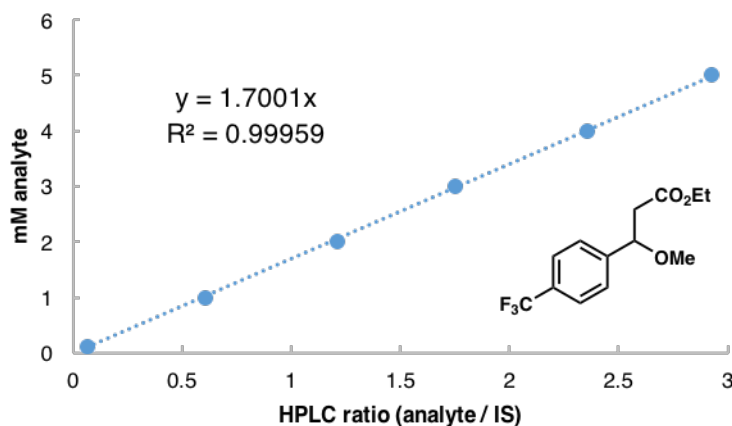
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm

Analysis data for **P411-CHF** catalyzed C–H alkylation of **1c** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3c -(1)	2433.4	2269.5	1.072	0.87	2.19	398		
3c -(2)	2583.9	2204.0	1.172	0.95	2.19	435		
3c -(3)	2728.2	2237.2	1.219	0.99	2.07	478		
3c -(4)	2024.0	2324.4	0.871	0.71	2.07	341	410	60

Ethyl 3-methoxy-3-(4-(trifluoromethyl)phenyl)propanoate (3d)

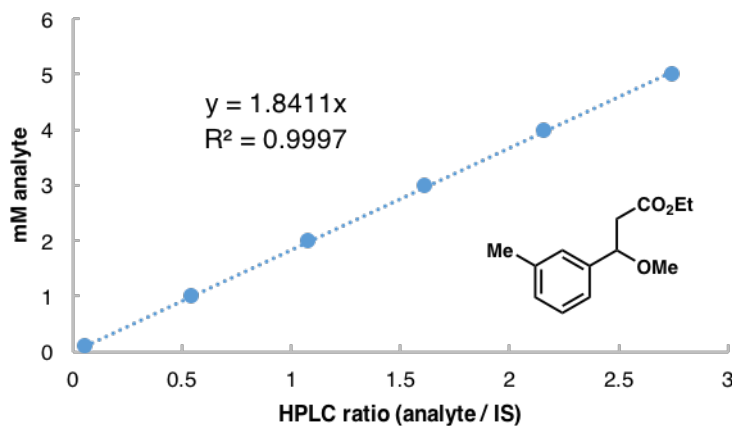
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm

Analysis data for **P411-CHF** catalyzed C–H alkylation of **1d** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3d -(1)	1689.4	2249.3	0.751	1.28	2.19	584	640	60
3d -(2)	1691.5	2250.5	0.752	1.28	2.19	585		
3d -(3)	1858.5	2192.0	0.848	1.44	2.07	697		
3d -(4)	1848.4	2229.5	0.829	1.41	2.07	681		

Ethyl 3-methoxy-3-(*m*-tolyl)propanoate (3e)

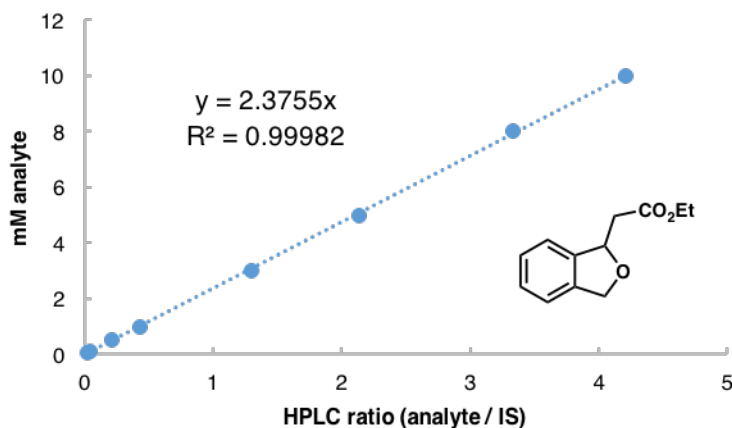
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm

Analysis data for **P411-CHF** catalyzed C–H alkylation of **1e** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3e -(1)	853.7	2313.9	0.369	0.68	1.84	370	390	30
3e -(2)	832.9	2311.4	0.360	0.66	1.84	361		
3e -(3)	1083.7	2335.0	0.464	0.85	2.06	416		
3e -(4)	1102.1	2327.5	0.474	0.87	2.06	424		

Ethyl 2-(1,3-dihydroisobenzofuran-1-yl)acetate (3f)

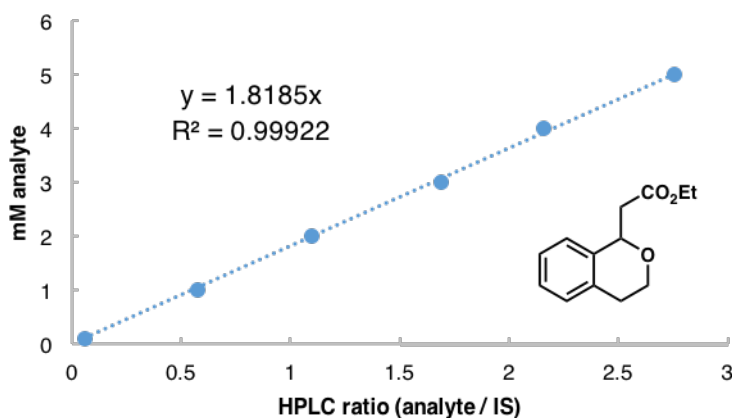
HPLC calibration curve with ethyl benzoate as internal standard (IS), at 220 nm

Analysis data for **P411-CHF** catalyzed C–H alkylation of **1f** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3f -(1)	1548.2	2209.2	0.701	1.66	1.84	906	900	10
3f -(2)	1530.2	2212.9	0.691	1.64	1.84	894		
3f -(3)	1737.5	2216.2	0.784	1.86	2.06	906		
3f -(4)	1716.1	2212.9	0.775	1.84	2.06	896		

Ethyl 2-(isochroman-1-yl)acetate (3g)

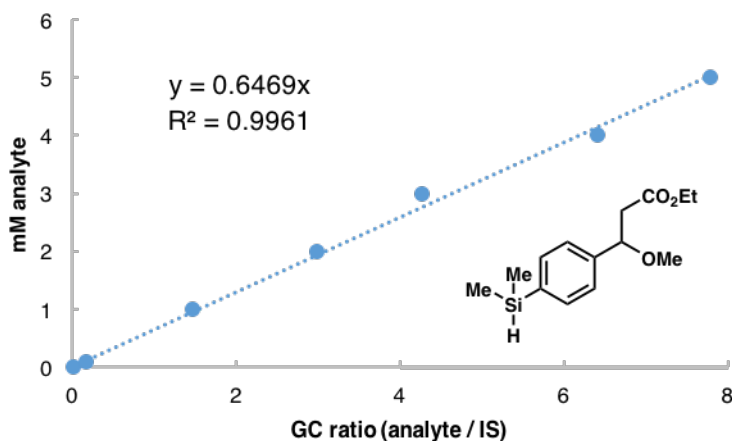
HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm

Analysis data for **P411-CHF** catalyzed C–H alkylation of **1g** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3g -(1)	2681.7	2293.4	1.169	2.13	1.84	1158	1210	80
3g -(2)	2646.0	2329.7	1.136	2.07	1.84	1125		
3g -(3)	3344.6	2355.0	1.420	2.58	2.06	1257		
3g -(4)	3388.4	2318.8	1.461	2.66	2.06	1293		

Ethyl 3-(4-(dimethylsilyl)phenyl)-3-methoxypropanoate (**3h**)

GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)

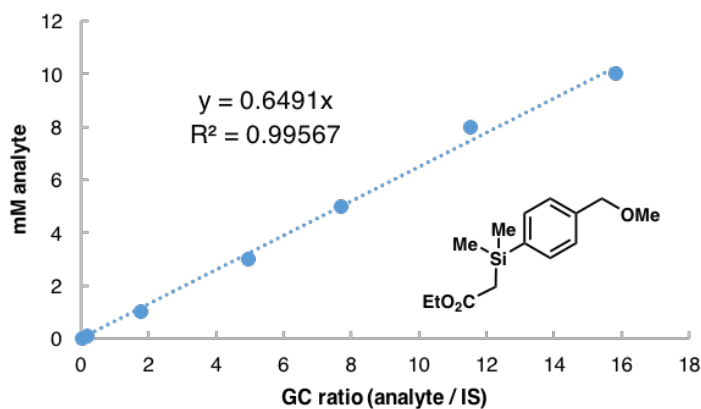


Analysis data for **P411-CHF** catalyzed C–H alkylation of **1h** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN	SD TTN
3h -(1)	2327.7	772.6	3.013	1.95	1.99	978		
3h -(2)	2193.5	729.1	3.009	1.95	1.99	977		
3h -(3)	2222.0	752.2	2.954	1.91	2.21	865		
3h -(4)	2288.8	755.6	3.029	1.96	2.21	887	930	60

Ethyl 2-((4-(methoxymethyl)phenyl)dimethylsilyl)acetate (**3h'**)

GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)



Analysis data for formation of **3h'** promoted by *E. coli* with **P411-CHF** (whole cells, room temp.):[†]

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/μM	Avg. [Pdt]/mM	SD [Pdt]/mM
3h' -(1)	2302.5	772.6	2.980	1.93	1.99		
3h' -(2)	2154.0	729.1	2.954	1.92	1.99		
3h' -(3)	2123.4	752.2	2.823	1.83	2.21		
3h' -(4)	2165.5	755.6	2.866	1.86	2.21	1.89	0.05

[†]Entries are from the same reactions as those used for analysis of **3h**. TTN is not calculated.

Analysis data for formation of **3h'** promoted by *E. coli* with **P-I263F** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	Avg. [Pdt]/mM	SD [Pdt]/mM
3h' -(5)	5975.7	826.8	7.228	4.69	2.47		
3h' -(6)	6069.1	848.6	7.152	4.64	2.47		
3h' -(7)	5927.5	842.4	7.036	4.57	2.50		
3h' -(8)	5974.8	823	7.260	4.71	2.50	4.65	0.06

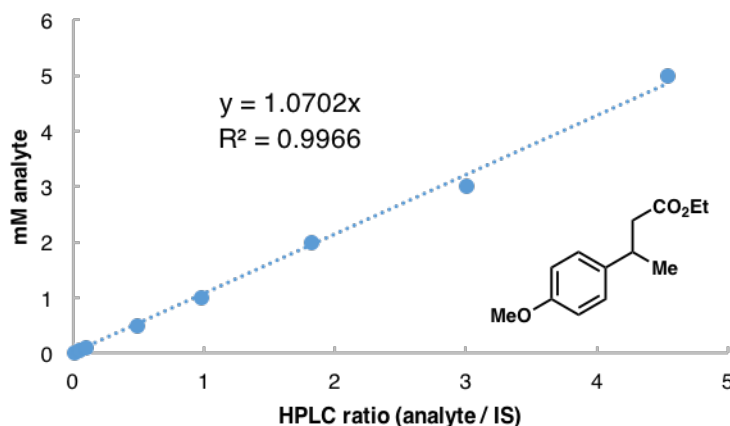
Analysis data for formation of **3h'** promoted by **vector control** *E. coli* (whole cells, room temp.):[†]

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M [#]	Avg. [Pdt]/mM	SD [Pdt]/mM
3h' -(9)	2939.1	847.9	3.466	2.25	0.77		
3h' -(10)	2704.2	823.7	3.283	2.13	0.77		
3h' -(11)	2933.7	865.3	3.390	2.20	0.87		
3h' -(12)	2893.1	823.2	3.514	2.28	0.87	2.22	0.07

Notes: [†]Vector control reactions employ *E. coli* cells that express a protein which does not contain a transition metal cofactor (halohydrin dehalogenase, UniProt ID: Q93D82). [#][PC] refers to the concentration of heme *b* measured by the hemochrome assay. Since a heme protein is not over-expressed in these cells, this is a measurement of free heme and/or native heme *b*-containing proteins found in the *E. coli*.

Ethyl 3-(4-methoxyphenyl)butanoate (**3i**)

HPLC calibration curve with ethyl benzoate as internal standard (IS), at 220 nm

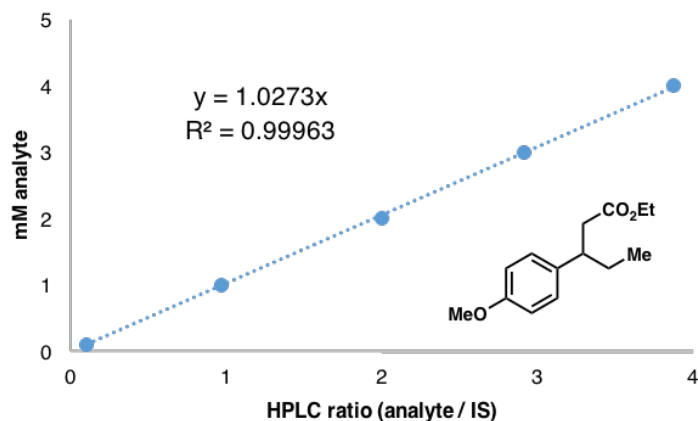


Analysis data for **P411-CHF** catalyzed C–H alkylation of **1i** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3i -(1)	2267.1	2277.4	0.995	1.07	2.02	527		
3i -(2)	2224.4	2284.4	0.974	1.04	2.02	515		
3i -(3)	2533.9	2294.2	1.104	1.18	2.24	528		
3i -(4)	2592.3	2297.6	1.128	1.21	2.24	540	530	10

Ethyl 3-(4-methoxyphenyl)pentanoate (**3j**)

HPLC calibration curve with ethyl phenoxyacetate as internal standard (IS), at 220 nm

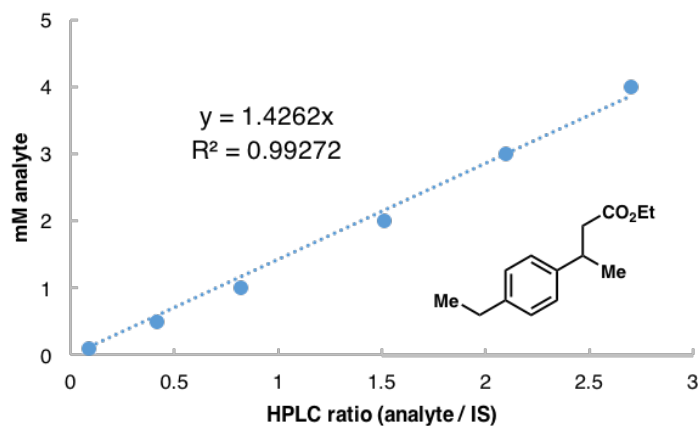


Analysis data for **P411-CHF** catalyzed C–H alkylation of **1j** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3j -(1)	192.6	2319.1	0.083	0.09	2.02	42		
3j -(2)	200.1	2285.5	0.088	0.09	2.02	44		
3j -(3)	236.0	2277.5	0.104	0.11	2.24	48		
3j -(4)	245.6	2308.5	0.106	0.11	2.24	49	46	3

Ethyl 3-(4-ethylphenyl)butanoate (**3k**)

HPLC calibration curve with ethyl benzoate as internal standard (IS), at 220 nm

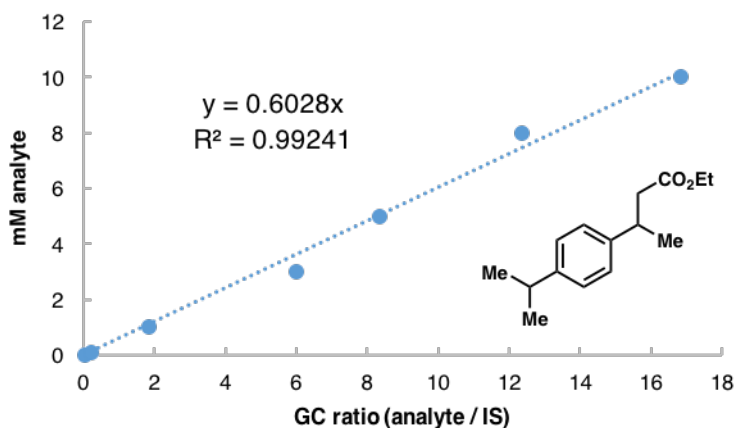


Analysis data for **P411-CHF** catalyzed C–H alkylation of **1k** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3k -(1)	970.6	2320.8	0.418	0.60	1.80	331		
3k -(2)	953.2	2327.4	0.410	0.58	1.80	324		
3k -(3)	1034.1	2412.6	0.429	0.61	1.78	343		
3k -(4)	1056.0	2426.4	0.435	0.62	1.78	348	340	10

Ethyl 3-(4-isopropylphenyl)butanoate (**3l**)

GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)

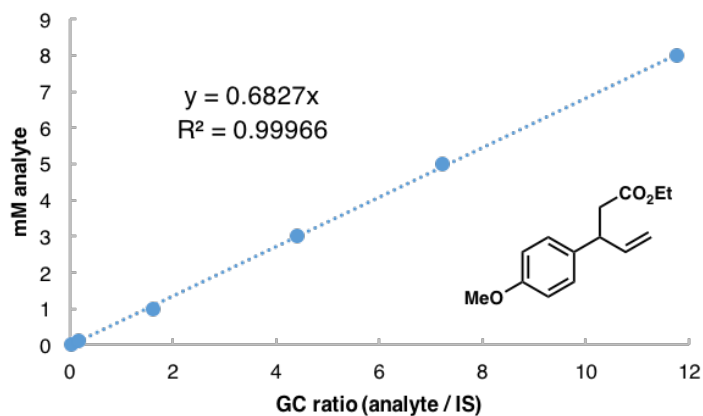


Analysis data for **P411-CHF** catalyzed C–H alkylation of **1l** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3l -(1)	354.6	765.1	0.463	0.28	1.99	140	140	5
3l -(2)	369.9	755.0	0.490	0.30	1.99	148		
3l -(3)	414.3	761.5	0.544	0.33	2.21	149		
3l -(4)	392.5	767.8	0.511	0.31	2.21	140		

Ethyl 3-(4-methoxyphenyl)pent-4-enoate (**3m**)

GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)

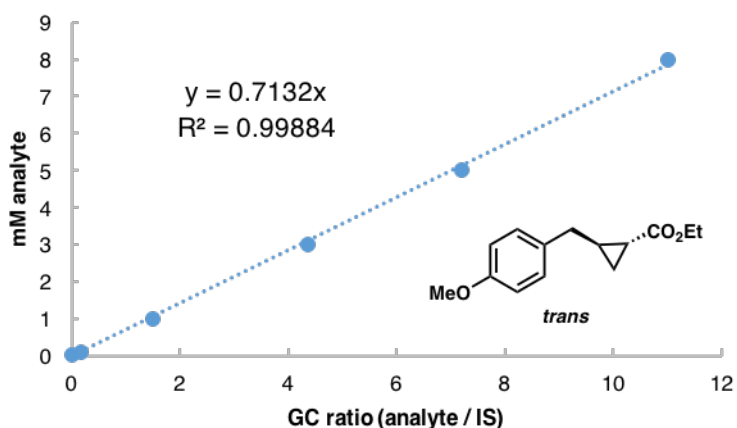
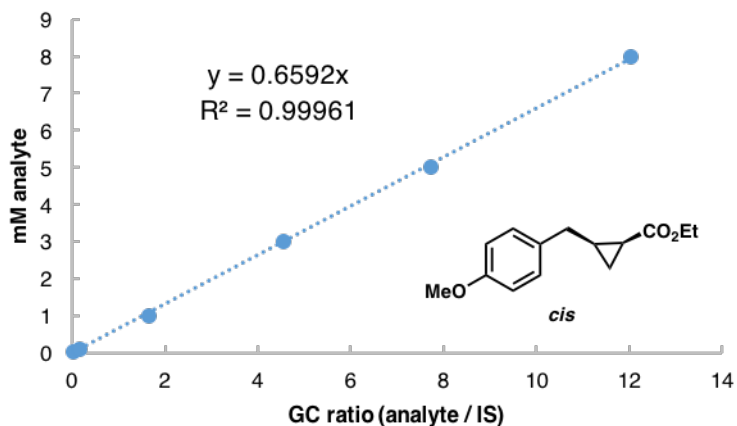


Analysis data for **P411-CHF** catalyzed C–H alkylation of **1m** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
3m -(1)	238.1	794.4	0.300	0.20	1.99	103	100	3
3m -(2)	252.2	797.3	0.316	0.22	1.99	108		
3m -(3)	262.0	784.7	0.334	0.23	2.21	103		
3m -(4)	254.9	753.3	0.338	0.23	2.21	105		

Ethyl 2-(4-methoxybenzyl)cyclopropane-1-carboxylate (**3m'**)

GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)



Analysis data for **P-I263F** catalyzed cyclopropanation of **1m** (whole cells, room temp.):

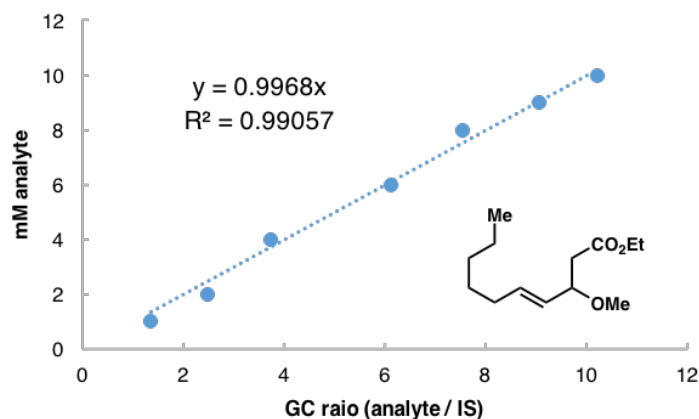
Pdt-Entries	Pdt (<i>cis</i>)	Pdt (<i>trans</i>)	IS	[Pdt]/mM*	[PC]/ μ M	TTN	d.r. [†]	Avg. TTN	SD TTN
3m' -(5)	556.9	359.4	887.9	0.702	2.47	284	59:41		
3m' -(6)	424.4	281.6	847.1	0.567	2.47	230	58:42		
3m' -(7)	590.1	379.0	826.2	0.798	2.50	319	59:41		
3m' -(8)	528.7	344.7	818.2	0.726	2.50	290	59:41	280	40

Notes: * Refers to the total concentration of cyclopropane products (*cis*-**3m'** + *trans*-**3m'**).

[†]Diastereomeric ratio (d.r.) is given as *cis* : *trans*.

Ethyl (*E*)-3-methoxydec-4-enoate (5a)

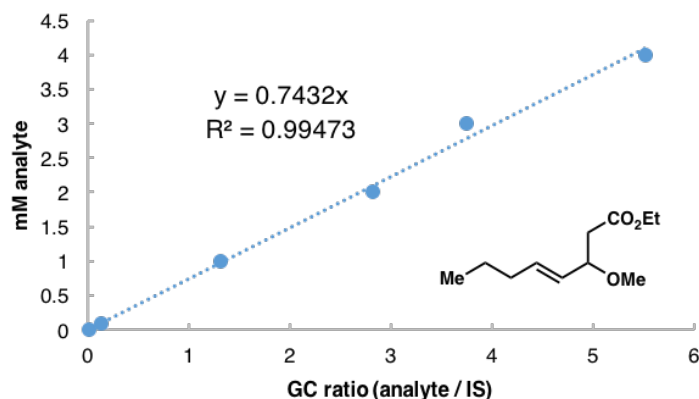
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)

Analysis data for **P411-CHF** catalyzed C–H alkylation of **4a** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
5a -(1)	1710.3	200.0	8.552	8.52	2.25	3789		
5a -(2)	1765.1	217.4	8.119	8.09	2.25	3597		
5a -(3)	1623.6	215.6	7.531	7.51	1.94	3879		
5a -(4)	1482.7	204.9	7.236	7.21	1.94	3727	3750	120

Ethyl (*E*)-3-methoxyoct-4-enoate (5b)

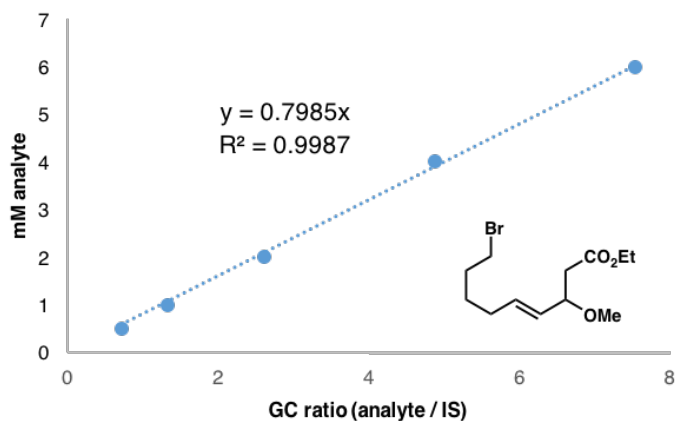
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)

Analysis data for **P411-CHF** catalyzed C–H alkylation of **4b** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
5b -(1)	2065.4	812.0	2.544	1.89	1.99	949		
5b -(2)	2036.8	795.5	2.560	1.90	1.99	955		
5b -(3)	2250.2	793.6	2.835	2.11	2.21	954		
5b -(4)	2217.2	790.8	2.804	2.08	2.21	944	950	5

Ethyl (*E*)-9-bromo-3-methoxynon-4-enoate (5c**)**

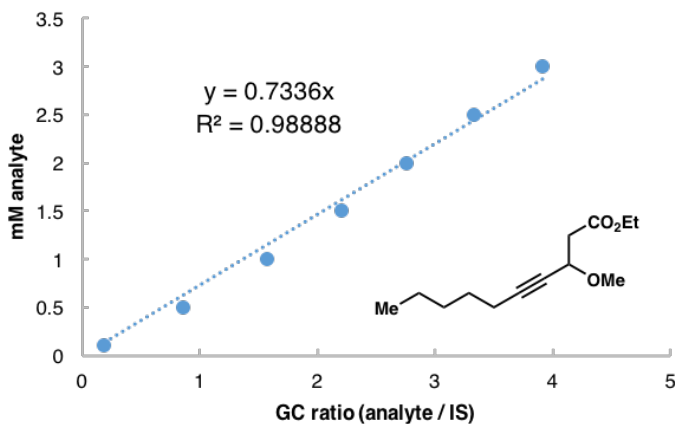
GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)

Analysis data for **P411-CHF** catalyzed C–H alkylation of **4c** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
5c -(1)	1071.0	179.5	5.967	4.76	1.80	2645		
5c -(2)	1062.7	178.6	5.950	4.75	1.80	2637		
5c -(3)	1142.9	175.3	6.520	5.21	1.78	2917		
5c -(4)	1140.9	180.4	6.324	5.05	1.78	2829	2760	140

Ethyl 3-methoxydec-4-ynoate (5e**)**

GC calibration curve with 1,3,5-trimethoxybenzene as internal standard (IS)

Analysis data for **P411-CHF** catalyzed C–H alkylation of **4e** (whole cells, room temp.):

Pdt-Entries	Pdt	IS	Pdt/IS	[Pdt]/mM	[PC]/ μ M	TTN	Avg. TTN	SD TTN
5e -(1)	405.4	903.5	0.449	0.33	1.80	183		
5e -(2)	370.3	856.6	0.432	0.32	1.80	176		
5e -(3)	388.3	843.5	0.460	0.34	1.78	189		
5e -(4)	407.2	828.8	0.491	0.36	1.78	202	190	10

IX. Enzymatic C–H alkylation reactions on preparative scale

Preparation of *E. coli* cells expressing P411 variants for preparative-scale reactions

HB_{amp} (480 mL) in a 1 L flask was inoculated with an overnight culture (20 mL, LB_{amp}) of *E. coli* (*E. coli* BL21(DE3)) cells containing a pET22b(+) plasmid encoding the desired P411 variant. The culture was shaken at 37 °C and 220 rpm (no humidity control) for 2.5 hours. The culture was placed on ice for 40 minutes, and 5-aminolevulinic acid (1.0 mM final concentration) and IPTG (0.5 mM final concentration) were added. The incubator temperature was reduced to 20 °C, and the culture was allowed to shake for 16–18 hours at 130 rpm. When greater amounts of cells were required, additional *E. coli* cultures were prepared in this manner. Cells were pelleted by centrifugation (3,000 × g, 5 min, 4 °C), resuspended in M9-N buffer and adjusted to OD₆₀₀ ≈ 60. An aliquot of cells at OD₆₀₀ ≈ 60 (3 mL) was taken for the hemochrome assay (see **Section I (G)**) to determine protein concentration. When applicable, the cell suspension was diluted with M9-N buffer to achieve the OD₆₀₀ which was used for the reaction. Cell suspensions in M9-N buffer were kept on ice until use.

General Procedure D: Enzymatic C–H alkylation reactions on preparative scale

Procedure D-I (alkane substrate is limiting reagent). To an Erlenmeyer flask equipped with a screw cap (reaction vessel, Chemglass CG-1543: 250 mL, 500 mL, or 1000 mL) was added a suspension of *E. coli* expressing the indicated P411 variant (generally OD₆₀₀ = 30). The headspace of the reaction vessel was degassed with argon (at least 1 hour for volumes less than 200 mL, at least 2 hours for volumes greater than 200 mL) while kept on ice. To degas the headspace of a flask containing *E. coli* cells, the flask is covered with aluminum foil and a stream of argon is flowed through the flask just above the cell suspension. Separately, a solution of *D*-glucose (250 mM in M9-N) was bubbled with argon and the headspace of a flask containing GOX oxygen depletion system (a solution of 14,000 U/mL catalase and 1,000 U/mL glucose oxidase, kept on ice) was degassed for at least 1 hour. In an anaerobic chamber, GOX, *D*-glucose, alkane substrate (1.0 equiv.), and diazo compound (1.0 equiv.) were added to the reaction vessel in this order. The vessel was capped, sealed with parafilm, and shaken (150–200 rpm) at room temperature. After one hour, the reaction vessel was transferred again to the anaerobic chamber where a second portion of *E. coli* cells expressing the P411 variant (the headspace of the flask containing these cells was degassed with argon following same procedure) and additional diazo compound (1.0 equiv.) were added. The vessel was capped, sealed with parafilm, and shaken (150–200 rpm) at room temperature for 14–17 additional hours. Final conditions were *E. coli* expressing P411 variant, alkane substrate (0.2–0.5 mmol, 1.0 equiv., larger scales for **3a**, **5a**, and **8f**, see **Section X**), diazo compound (2.0 equiv.), *D*-glucose (25 mM), GOX oxygen depletion system (700 U/mL catalase, 50 U/mL glucose oxidase), 2 vol% EtOH in M9-N buffer under anaerobic conditions; total reaction time 15–18 hours.

Procedure D-II (diazo compound is limiting reagent). To an Erlenmeyer flask equipped with a screw cap (reaction vessel, Chemglass CG-1543: 250 mL, 500 mL, or 1000 mL) was added a suspension of *E. coli* expressing the indicated P411 variant. The headspace of the reaction vessel was degassed with argon (at least 1 hour) while kept on ice. To degas the headspace of a flask containing *E. coli* cells, the flask is covered with aluminum foil and a stream of argon is flowed through the flask just above the cell suspension. Separately, a solution of *D*-glucose (250 mM in M9-N) was bubbled with argon. In an anaerobic chamber, *D*-glucose, alkane substrate (2.0 equiv.),

and diazo compound (1.0 equiv.) were added to the reaction vessel in this order. The vessel was capped, sealed with parafilm, and shaken (150–200 rpm) at room temperature. Final conditions were *E. coli* expressing P411 variant, alkane substrate (1.0 mmol, 2.0 equiv.), diazo compound (0.5 mmol, 1.0 equiv.), *D*-glucose (25 mM), 2 vol% EtOH in M9-N buffer under anaerobic conditions; total reaction time 15–18 hours.

Workup Procedure D-i. Every 35 mL portion of the preparative scale reaction mixture was transferred to a 50 mL Eppendorf conical tube (catalog no. 0030122178). To the reaction mixture in every tube was added 15 mL mixed organic solvent (1 : 1 hexanes : ethyl acetate); the solution was shaken vigorously and centrifuged ($10,000 \times g$, 5 minutes, Beckman-Coulter Avanti J-25 centrifuge equipped with JA-12 rotor) to separate the organic and aqueous layers. The organic layer was collected and the aqueous layer was subject to three additional rounds of extraction. The organic layers were combined, dried over Na_2SO_4 , and concentrated under reduced pressure. Purification was performed by silica column chromatography with either hexanes / ethyl acetate or hexanes / dichloromethane / diethyl ether as eluent systems to afford the desired product. Additional purification by reverse phase chromatography (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / methanol eluent system) was utilized if necessary. TTNs were calculated based on measured protein concentration and the isolated yield of the product.

Workup Procedure D-ii. Every ~100–125 mL portion of preparative scale reaction mixture was transferred to a centrifuge bottle. To the reaction mixture in every bottle was added equal volume ethyl acetate; the solution was shaken vigorously and centrifuged ($14,000 \times g$, 10 minutes, Beckman-Coulter Avanti J-25 centrifuge equipped with JA-10 rotor) to separate the organic and aqueous layers. The organic layer was collected and the aqueous layer was subject to three additional rounds of extraction. The combined organics were dried over Na_2SO_4 , and concentrated under reduced pressure. Purification was performed by silica column chromatography with either hexanes / ethyl acetate or hexanes / dichloromethane / diethyl ether as eluent systems to afford the desired product. Additional purification by reverse phase chromatography (Biotage Isolera equipped with Biotage SNAP Ultra C18 column, water / methanol eluent system) was utilized if necessary. TTNs were calculated based on measured protein concentration and the isolated yield of the product.

Ethyl 3-methoxy-3-(4-methoxyphenyl)propanoate (3a)

This compound was prepared using a modified version of **General Procedure D-I** carried out at 4 °C. The reaction mixture was kept on ice during the addition of all reagents and shaken in an incubator set to 4 °C and 150 rpm. In addition, a solution of $\text{Na}_2\text{S}_2\text{O}_4$ (5 mL, 40 mM in M9-N, bubbled with argon for 1 hour) was added following the second addition of *E. coli* cells expressing P411-CHF. Final conditions were *E. coli* expressing P411-CHF (resuspended to $\text{OD}_{600} = 44$), alkane substrate (1.0 mmol, 1.0 equiv.), ethyl diazoacetate (2.0 mmol, 2.0 equiv.), *D*-glucose (25 mM), GOX oxygen depletion system (700 U/mL catalase, 50 U/mL glucose oxidase), $\text{Na}_2\text{S}_2\text{O}_4$ (1 mM), 2 vol% EtOH in M9-N buffer under anaerobic conditions at 4 °C; total reaction time 18 hours.

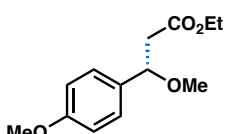
The reaction was quenched with the addition of 100 mL acetonitrile. The crude reaction mixture was transferred to a centrifuge tube, shaken vigorously, and centrifuged to pellet the cells ($14,000 \times g$, 10 minutes). The supernatant was decanted and the acetonitrile was removed under reduced pressure. Following, the aqueous layer was extracted with ethyl acetate (3×100 mL). The

cell pellet was resuspended in H₂O and this suspension was also extracted using ethyl acetate (3 × 20 mL; centrifugation (3,000 × g, 5 min) was used to help separate the organic and aqueous layers. The combined organics were dried over Na₂SO₄ and concentrated under reduced pressure. Purification by reverse phase preparative HPLC (column: Eclipse XDB-C8, 5 μm, 9.4 × 250 mm) using water / acetonitrile eluent system afforded **3a**.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD ₆₀₀ = 44, 4 °C)				GOX solution ^a	<i>D</i> -glucose in M9-N ^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
110.0	51.0 [*]	4.77	0.768	10.0	20.0
Alkane substrate (1a) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.50	2.0	1.0	1.0	1.0; 1.0	2.0
Purification eluent		Product			
1: water / MeCN (reverse phase preparative HPLC)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
		194.7	0.817	82%	1060

*A solution of Na₂S₂O₄ (5 mL, 40 mM in M9-N, bubbled with argon for 1 hour) was added following the second addition of *E. coli* cells expressing P411-CHF. Final concentration of Na₂S₂O₄ in the reaction is 1 mM.

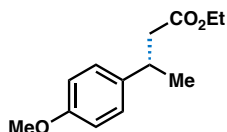
Notes: [PC] = protein concentration in original cell suspension, n_pro = amount of protein in the reaction, n_1 = amount of alkane substrate in the reaction, n_2 = total amount of diazo compound in the reaction, add. = addition, m[Pdt] = mass of product isolated, n[Pdt] = amount of product. ^a GOX oxygen depletion system is 14,000 U/mL catalase and 1,000 U/ mL glucose oxidase in 0.1 M potassium phosphate buffer (pH=8.0); the final reaction mixture contains 700 U/mL catalase and 50 U/mL glucose oxidase. ^b *D*-glucose stock solution is 250 mM in M9-N buffer; final concentration of *D*-glucose in the reaction is 25 mM. These notes apply for all tables in this section.

 ¹H NMR (400 MHz, CDCl₃) δ 7.25 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 4.58 (dd, *J* = 9.0, 4.9 Hz, 1H), 4.14 (qd, *J* = 7.1, 1.2 Hz, 2H), 3.81 (s, 3H), 3.19 (s, 3H), 2.80 (dd, *J* = 15.2, 9.0 Hz, 1H), 2.55 (dd, *J* = 15.2, 4.9 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.2, 159.5, 132.7, 128.0, 114.0, 79.7, 60.7, 56.7, 55.4, 43.7, 14.3. HRMS (EI) *m/z*: 238.1213 (*M*⁺); calc. for C₁₃H₁₈O₄: 238.1205. [α]_D²³ = −46.354 ± 0.411° (*c* 0.5, CHCl₃). SFC Chiralpak AD-H column (3% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C), *t*_r = 9.02 min (major), 10.50 min (minor), 98.0 : 2.0 e.r.

Ethyl 3-(4-methoxyphenyl)butanoate (3i)

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*i*.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD ₆₀₀ = 29)				GOX solution ^a	<i>D</i> -glucose in M9-N ^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
110.0	56.0	2.25	0.374	10.0	20.0
Alkane substrate (1i) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.10	2.0	0.2	0.20	1.0; 1.0	0.4
Purification eluent		Product			
1: Hex / EtOAc (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
2: water / MeOH (reverse phase)		21.3	0.096	48%	260

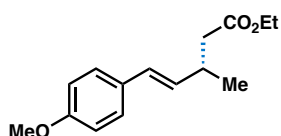


Spectral data is in agreement with literature report⁴⁰. ¹H NMR (500 MHz, CDCl₃) δ 7.14 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.08 (qd, *J* = 7.2, 1.2 Hz, 2H), 3.79 (s, 3H), 3.24 (h, *J* = 7.1 Hz, 1H), 2.57 (dd, *J* = 14.9, 7.2 Hz, 1H), 2.51 (dd, *J* = 14.9, 8.0 Hz, 1H), 1.28 (d, *J* = 7.0 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 172.6, 158.2, 138.0, 127.8, 113.9, 60.4, 55.4, 43.4, 35.9, 22.1, 14.3. [α]_D²³ = +26.334 ± 0.676° (*c* 0.5, CHCl₃). SFC Chiralcel OB-H column (supercritical CO₂, 2.5 mL/min, 40 °C), *t*_r = 5.50 min (minor), 6.24 min (major), 97.9 : 2.1 e.r.

Ethyl (*E*)-5-(4-methoxyphenyl)-3-methylpent-4-enoate (5d)

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*ii*.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD ₆₀₀ = 32)				GOX solution ^a	<i>D</i> -glucose in M9-N ^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
137.5	70.0	2.59	0.536	12.5	25.0
Alkane substrate (4d) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.10	2.5	0.25	0.20	1.25; 1.25	0.50
Purification eluent		Product			
Hex / (4 : 1 DCM : Et ₂ O) (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
		9.7	0.039	15.6%	70

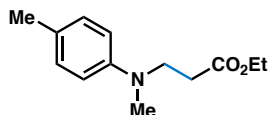


¹H NMR (400 MHz, CDCl₃) δ 7.30 – 7.24 (m, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.34 (d, *J* = 15.9 Hz, 1H), 5.99 (dd, *J* = 15.9, 7.6 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.80 (s, 3H), 2.90 – 2.75 (m, 1H), 2.41 (dd, *J* = 14.7, 7.3 Hz, 1H), 2.34 (dd, *J* = 14.7, 7.3 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.14 (d, *J* = 6.7 Hz, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 172.6, 159.0, 132.3, 130.4, 128.3, 127.3, 114.0, 60.4, 55.4, 42.1, 34.3, 20.5, 14.5. **HRMS** (FAB) *m/z*: 248.1417 (*M*⁺); calc. for C₁₅H₂₀O₃: 248.1413. **SFC Chiralcel OB-H column** (3% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C), *t_r* = 6.62 min (minor), 7.66 min (major), 97.0 : 3.0 e.r.

Ethyl 3-(methyl(*p*-tolyl)amino)propanoate (8a)

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*i*.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD ₆₀₀ = 29)				GOX solution ^a	<i>D</i> -glucose in M9-N ^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
55.0	28.0	2.13	0.177	5.0	10.0
Alkane substrate (7a) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.50	1.0	0.50	1.00	0.50; 0.50	1.0
Purification eluent		Product			
1: Hex / EtOAc (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
		91.2	0.412	82%	2330

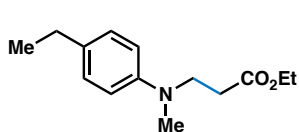


Spectral data is in agreement with literature report⁴³. **¹H NMR** (400 MHz, CDCl₃) δ 7.05 (d, *J* = 8.3 Hz, 2H), 6.67 (d, *J* = 8.6 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.64 (t, *J* = 7.2 Hz, 2H), 2.90 (s, 3H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.25 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 172.6, 146.8, 129.9, 126.3, 113.1, 60.7, 49.1, 38.5, 31.8, 20.4, 14.3.

Ethyl 3-((4-ethylphenyl)(methyl)amino)propanoate (8b)

This compound was prepared using **General Procedure D-I** and Workup Procedure D-ii.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD ₆₀₀ = 30)				GOX solution ^a	<i>D</i> -glucose in M9-N ^a
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
55.0	28.0	2.02	0.168	5.0	10.0
Alkane substrate (7b) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.50	1.0	0.50	1.00	0.50; 0.50	1.0
Purification eluent		Product			
1: Hex / (4 : 1 DCM : Et ₂ O) (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
2: water / MeOH (reverse phase)		88.0	0.374	75%	2230

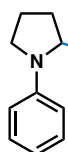


¹H NMR (400 MHz, CDCl₃) δ 7.08 (d, *J* = 8.7 Hz, 2H), 6.69 (d, *J* = 8.8 Hz, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.65 (t, *J* = 7.2 Hz, 2H), 2.91 (s, 3H), 2.60 – 2.51 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.20 (t, *J* = 7.6 Hz, 3H).
¹³C NMR (101 MHz, CDCl₃) δ 172.6, 146.9, 132.8, 128.7, 113.0, 60.7, 49.1, 38.5, 31.9, 27.9, 16.1, 14.3. **HRMS** (ESI-TOF) *m/z*: 236.1673 (*M* + *H*⁺); calc. for C₁₄H₂₂NO₂: 236.1651.

Ethyl 2-(1-phenylpyrrolidin-2-yl)acetate (8c)

This compound was prepared using **General Procedure D-I** and Workup Procedure D-ii.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD ₆₀₀ = 31)				GOX solution ^a	<i>D</i> -glucose in M9-N ^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
55.0	28.0	2.65	0.220	5.0	10.0
Alkane substrate (7c) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.50	1.0	0.50	1.00	0.50; 0.50	1.0
Purification eluent		Product			
1: Hex / (4 : 1 DCM : Et ₂ O) (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
		104.1	0.446	89%	2030

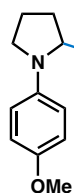


¹H NMR (400 MHz, CDCl₃) δ 7.29 – 7.20 (m, 2H), 6.69 (tt, *J* = 7.2, 1.1 Hz, 1H), 6.62 (d, *J* = 7.9 Hz, 2H), 4.21 – 4.14 (m, 3H), 3.48 – 3.37 (m, 1H), 3.23 – 3.14 (m, 1H), 2.79 (dd, *J* = 15.0, 2.9 Hz, 1H), 2.22 (dd, *J* = 15.0, 10.5 Hz, 1H), 2.12 – 1.98 (m, 3H), 1.97 – 1.82 (m, 1H), 1.29 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 172.2, 146.6, 129.5, 116.0, 112.0, 60.6, 55.5, 48.0, 37.8, 31.1, 23.1, 14.4. HRMS (ESI-TOF) *m/z*: 234.1491 (*M* + *H*⁺); calc. for C₁₄H₂₀NO₂: 234.1494. [α]_D²³ = +2.056 ± 0.834° (*c* 0.5, CHCl₃). HPLC Chiralcel OD-H column (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, room temperature), *t*_r = 6.20 min (minor), 8.58 min (major), 82.8 : 17.2 e.r.

Ethyl 2-(1-(4-methoxyphenyl)pyrrolidin-2-yl)acetate (8d)

This compound was prepared using General Procedure D-I and Workup Procedure D-i.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD ₆₀₀ = 31)				GOX solution ^a	<i>D</i> -glucose in M9-N ^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n _{pro} /μmol	volume/mL	volume/mL
55.0	28.0	2.17	0.180	5.0	10.0
Alkane substrate (7d) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n ₁ /mmol	stock/M	volume/mL add. 1; add. 2	n ₂ /mmol
0.50	1.0	0.50	1.0	0.50; 0.50	1.0
Purification eluent		Product			
1: Hex / EtOAc (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
2: water / MeOH (reverse phase)		102.1	0.388	78%	2150

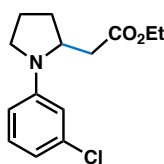


¹H NMR (400 MHz, CDCl₃) δ 6.86 (d, *J* = 9.1 Hz, 2H), 6.58 (d, *J* = 9.1 Hz, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 4.13 – 4.05 (m, 1H), 3.76 (s, 3H), 3.42 – 3.35 (m, 1H), 3.17 – 3.09 (m, 1H), 2.76 (dd, *J* = 14.9, 3.0 Hz, 1H), 2.20 (dd, *J* = 14.9, 10.4 Hz, 1H), 2.13 – 1.96 (m, 3H), 1.92 – 1.82 (m, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 172.3, 151.1, 141.5, 115.3, 112.9, 60.6, 56.1, 56.0, 48.6, 38.1, 31.1, 23.3, 14.4. HRMS (FAB) *m/z*: 263.1518 (*M*⁺); calc. for C₁₅H₂₁NO₃: 263.1521. [α]_D²³ = +7.310 ± 0.478° (*c* 0.5, CHCl₃). HPLC Chiralcel OD-H column (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, room temperature), *t*_r = 7.25 min (minor), 8.12 min (major), 83.7 : 16.3 e.r.

Ethyl 2-(1-(3-chlorophenyl)pyrrolidin-2-yl)acetate (8e)

This compound was prepared using **General Procedure D-I** and Workup Procedure D-i.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD₆₀₀ = 29)				GOX solution^a	<i>D</i>-glucose in M9-N^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
137.5	70.0	3.24	0.673	12.5	25.0
Alkane substrate (7e) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.20	2.5	0.50	0.40	1.25; 1.25	1.0
Purification eluent		Product			
1: Hex / EtOAc (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
2: water / MeOH (reverse phase)		89.6	0.335	67%	500



¹H NMR (500 MHz, CDCl₃) δ 7.13 (t, *J* = 8.1 Hz, 1H), 6.65 (dd, *J* = 7.9, 1.8 Hz, 1H), 6.57 (t, *J* = 2.2 Hz, 1H), 6.48 (dd, *J* = 8.3, 2.3 Hz, 1H), 4.23 – 4.12 (m, 3H), 3.44 – 3.36 (m, 1H), 3.22 – 3.12 (m, 1H), 2.74 (dd, *J* = 15.1, 3.0 Hz, 1H), 2.23 (dd, *J* = 15.1, 10.4 Hz, 1H), 2.13 – 2.01 (m, 3H), 1.97 – 1.85 (m, 1H), 1.30 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 171.9, 147.6, 135.3, 130.4, 115.9, 111.9, 110.2, 60.7, 55.5, 48.1, 37.7, 31.1, 23.0, 14.4. HRMS (ESI-TOF) *m/z*: 268.1094 (*M* + H⁺); calc. for C₁₄H₁₉NO₂³⁵Cl: 268.1104. [α]_D²³ = +7.144 ± 0.875° (*c* 0.5, CHCl₃). HPLC Chiralcel OD-H column (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, room temperature), *t*_r = 6.29 min (minor), 6.87 min (major), 90.3 : 9.7 e.r.

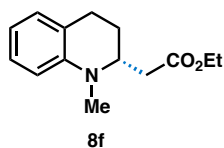
(–)-Ethyl (*R*)-2-(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)acetate ((–)-8f)

This compound was prepared using **General Procedure D-I** and Workup Procedure D-i.

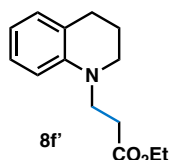
<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD₆₀₀ = 31)				GOX solution^a	<i>D</i>-glucose in M9-N^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
55.0	28.0	2.40	0.199	5.0	10.0
Alkane substrate (7f) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.50	1.0	0.50	1.00	0.50; 0.50	1.0
Purification eluent		Product			
1: Hex / (4 : 1 DCM : Et ₂ O) (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
2: water / acetonitrile (reverse phase preparative HPLC)		48.9 ^c	0.210	42%	1050 ^d

^c Regiomer ratio 9 : 1 for **8f** : **8f'** determined by ¹H NMR.

^d Reported for the sum of regioisomers **8f** and **8f'**.



9 : 1 r.r. (8f : 8f')



¹H NMR (400 MHz, CDCl₃) δ 7.14 – 7.02 (m, 1H, both isomers), 6.99 (d, *J* = 7.3 Hz, 0.9H, isomer **8f**), 6.97 – 6.93 (m, 0.1H, isomer **8f'**), 6.68 – 6.51 (m, 2H, both isomers), 4.15 (q, *J* = 7.1 Hz, 2H, both isomers), 3.86 – 3.78 (m, 0.9H, isomer **8f**), 3.65 – 3.57 (m, 0.2H, isomer **8f'**), 3.32 – 3.25 (m, 0.2H, isomer **8f'**), 2.93 (s, 2.7H, isomer **8f**), 2.91 – 2.79 (m, 0.9H, isomer **8f**), 2.77 – 2.67 (m, 1.1H, both isomers), 2.65 – 2.54 (m, 1.1H, both isomers), 2.39 (dd, *J* = 14.7, 8.6 Hz, 0.9H, isomer **8f**), 2.08 – 1.84 (m, 2H, both isomers), 1.30 – 1.24 (m, 3H, both isomers). ¹³C NMR (101 MHz, CDCl₃) δ 172.6, 172.3, 144.8, 144.6, 129.5, 129.0, 127.3, 127.3, 122.8, 121.6, 116.2, 116.2, 111.0, 110.7, 60.7, 60.7, 56.1, 49.6, 47.3, 37.8, 36.7, 31.5, 28.2, 25.5, 23.3, 22.3, 14.4. With variant **P411-CHF**: [α]_D²³ = −2.980 ± 0.898° (*c* 1.0, CHCl₃). With variant **P411-CHF**: SFC Chiralcel OB-H column (3% *i*-PrOH supercritical CO₂, 2.5 mL/min, 40 °C), *t*_r = 6.47 min (major), 7.38 min (minor), 73.0 : 27.0 e.r.

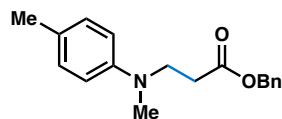
Benzyl 3-(methyl(*p*-tolyl)amino)propanoate (**10a**)

This compound was prepared using **General Procedure D-I** and Workup Procedure D-*i*.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF , OD ₆₀₀ = 30)				GOX solution ^a	<i>D</i> -glucose in M9-N ^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
55.0	28.0	2.04	0.170	5.0	10.0
Alkane substrate (7a) stock in EtOH			Diazo (9a) stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.50	1.0	0.50	1.00	0.50; 0.50	1.0
Purification eluent		Product			
1 : Hex / EtOAc (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
		19.0 ^c	0.061 ^d	12% ^d	360 ^d

^c Isolated with 10% diazo dimer.

^d Corrected for diazo dimer.

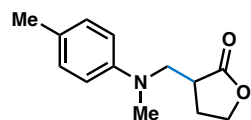


This compound is known in the literature.⁴⁴ ¹H NMR (400 MHz, CDCl₃) δ 7.39 – 7.31 (m, 5H), 7.04 (d, *J* = 8.7 Hz, 2H), 6.66 (d, *J* = 8.8 Hz, 2H), 5.11 (s, 2H), 3.66 (t, *J* = 7.2 Hz, 2H), 2.87 (s, 3H), 2.61 (t, *J* = 7.2 Hz, 2H), 2.25 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 172.4, 146.7, 135.9, 133.9, 129.9, 128.7, 128.4, 126.3, 113.2, 66.5, 49.1, 38.5, 31.8, 20.4. HRMS (ESI-TOF) *m/z*: 284.1635 (M + H⁺); calc. for C₁₈H₂₂NO₂: 284.1651.

3-((Methyl(*p*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (10b)

This compound was prepared using **General Procedure D-II** and Workup Procedure D-*i*.

<i>E. coli</i> suspension in M9-N (variant: P411-IY (T327I), OD ₆₀₀ = 55)			GOX solution ^a	<i>D</i> -glucose in M9-N ^b	
Volume/ mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL	
88.0	6.77	0.595	0.0	10.0	
Alkane substrate (7a) stock in EtOH		Diazo (9b) stock in EtOH			
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL	n_2/mmol
1.0	1.0	1.0	0.50	1.0	0.50
Purification eluent		Product			
1: Hex / EtOAc (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
		37.2	0.170	34%	280

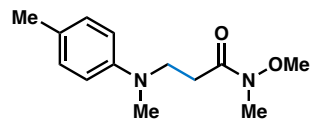


¹H NMR (400 MHz, CDCl₃) δ 7.07 (d, *J* = 8.3 Hz, 2H), 6.68 (d, *J* = 8.6 Hz, 2H), 4.35 (td, *J* = 8.8, 2.6 Hz, 1H), 4.15 (ddd, *J* = 10.0, 9.1, 6.6 Hz, 1H), 3.94 (dd, *J* = 15.1, 4.5 Hz, 1H), 3.43 (dd, *J* = 15.1, 8.0 Hz, 1H), 2.96 (s, 3H), 2.91 (dddd, *J* = 10.6, 8.6, 8.0, 4.5 Hz, 1H), 2.34 (dddd, *J* = 12.7, 8.9, 6.6, 2.6 Hz, 1H), 2.26 (s, 3H), 2.11 (dtd, *J* = 12.8, 10.2, 8.5 Hz, 1H). ¹³C NMR (101 MHz, CDCl₃) δ 178.5, 146.8, 130.0, 126.7, 113.1, 66.7, 53.3, 39.6, 38.7, 28.1, 20.4. HRMS (FAB) *m/z*: 218.1172 ([*M* + H⁺) – H₂]; calc. for C₁₃H₁₆NO₂: 218.1181. With variant P411-IY T327I: HPLC Chiralcel OD-H column (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, 32 °C), *t_r* = 22.826 min (major), 24.286 min (minor), 78.0 : 22.0 e.r.

N-Methoxy-*N*-methyl-3-(methyl(*p*-tolyl)amino)propanamide (10c)

This compound was prepared using **General Procedure D-II** and Workup Procedure D-*i*.

<i>E. coli</i> suspension in M9-N (variant: P411-IY (T327I), OD ₆₀₀ = 55)			GOX solution ^a	<i>D</i> -glucose in M9-N ^b	
Volume/ mL		[PC]/μM	n _{pro} /μmol	volume/mL	volume/mL
88.0		7.00	0.616	0.0	10.0
Alkane substrate (7a) stock in EtOH			Diazo (9c) stock in EtOH		
stock/M	volume/mL	n ₁ /mmol	stock/M	volume/mL	n ₂ /mmol
1.0	1.0	1.0	0.50	1.0	0.50
Purification eluent		Product			
1: Hex / EtOAc (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
		72.3	0.306	61%	500



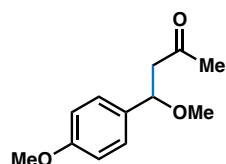
¹H NMR (400 MHz, CDCl₃) δ 7.04 (d, *J* = 8.2 Hz, 2H), 6.68 (d, *J* = 8.7 Hz, 2H), 3.70 – 3.64 (m, 2H), 3.62 (s, 3H), 3.17 (s, 3H), 2.92 (s, 3H), 2.66 (t, *J* = 7.2 Hz, 2H), 2.25 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 173.2, 146.8, 129.9, 125.9, 112.8, 61.5, 48.8, 38.6, 32.2, 29.0, 20.4. HRMS (ESI-TOF) *m/z*: 237.1616 (*M* + H⁺); calc. for C₁₃H₂₁N₂O₂: 237.1603.

4-Methoxy-4-(4-methoxyphenyl)butan-2-one (10d)

This compound was prepared using **General Procedure D-II** and Workup Procedure D-*i*.

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD ₆₀₀ = 60) ^c			GOX solution ^a	<i>D</i> -glucose in M9-N ^b	
Volume/ mL		[PC]/μM	n _{pro} /μmol	volume/mL	volume/mL
88.0		8.17	0.719	0.0	10.0
Alkane substrate (1a) stock in EtOH			Diazo (9d) stock in EtOH		
stock/M	volume/mL	n ₁ /mmol	stock/M	volume/mL	n ₂ /mmol
1.0	1.0	1.0	0.50	1.0	0.50
Purification eluent		Product			
1 : Hex / EtOAc (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
		21.8	0.105	21%	150

^c Enzyme was expressed using a modified procedure. After addition of 5-aminolevulinic acid (1.0 mM final concentration) and IPTG (0.5 mM final concentration), the incubator temperature was set to 24 °C, and the culture was allowed to shake for 20 hours at 140 rpm. Product formation was also observed when cultures were expressed following the typical protocol.



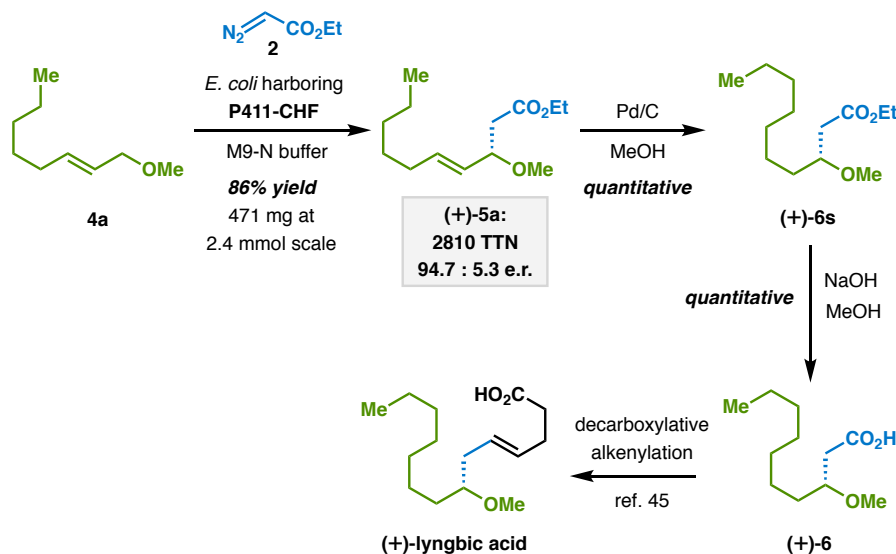
¹H NMR (400 MHz, CDCl₃) δ 7.24 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.58 (dd, *J* = 8.8, 4.5 Hz, 1H), 3.81 (s, 3H), 3.17 (s, 3H), 2.97 (dd, *J* = 15.8, 8.8 Hz, 1H), 2.58 (dd, *J* = 15.8, 4.5 Hz, 1H), 2.15 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 206.9, 159.4, 133.0, 127.9, 114.1, 79.3, 56.6, 55.4, 52.0, 31.2.

HRMS (ESI-TOF) *m/z*: 231.0999 (*M* + Na⁺); calc. for C₁₂H₁₆O₃Na: 231.0997.

HPLC Chiralcel OJ-H column (6% *i*-PrOH in *n*-hexane, 1.0 mL/min, 28 °C), *t_r* = 20.152 min (major), 21.760 min (minor), 71.0 : 29.0 e.r.

X. Syntheses of (+)-lyngbic acid and (+)-cuspareine

Experimental details for the formal synthesis of (*R*)-(+)-lyngbic acid and the total synthesis of (*R*)-(+)-cuspareine can be found below. Enzymatic C–H alkylation is the key stereo-defining step in the routes to both molecules.



Supplementary Fig. 14. Detailed scheme for the formal synthesis of (+)-lyngbic acid.

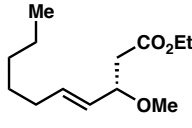
(+)-Ethyl (*E*)-3-methoxydec-4-enoate ((+)-5a)

Prepared following **General Procedure D-I** and Workup Procedure D-ii (see **Section IX**).

<i>E. coli</i> suspension in M9-N (variant: P411-CHF, OD ₆₀₀ = 29)				GOX solution ^a	<i>D</i> -glucose in M9-N ^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
220.0	112.0	2.21	0.734	20.0	40.0
Alkane substrate (4a) stock in EtOH			Ethyl diazoacetate (2) stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.60	4.0	2.4	1.2	2.0; 2.0	4.8
Purification eluent		Product			
1: Hex / (4 : 1 DCM : Et ₂ O) (normal phase)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
2: water / MeOH (reverse phase)		470.6	2.061	86%	2810

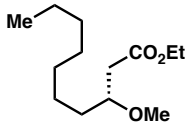
Notes: [PC] = protein concentration in original cell suspension, n_pro = amount of protein in the reaction, n_1 = amount of alkane substrate in the reaction, n_2 = total amount of diazo compound in the reaction, add. = addition, m[Pdt] = mass of product isolated, n[Pdt] = amount of product. ^a GOX oxygen depletion system is 14,000 U/mL catalase and 1,000 U/ mL glucose oxidase in 0.1 M potassium phosphate buffer (pH=8.0); the final reaction mixture contains 700 U/mL catalase and 50 U/mL glucose oxidase. ^b *D*-glucose stock solution is 250 mM; final concentration of *D*-

glucose in the reaction is 25 mM. These notes apply for all tables in this section.

 **¹H NMR** (400 MHz, CDCl₃) δ 5.69 (dt, *J* = 15.4, 6.8 Hz, 1H), 5.28 (ddt, *J* = 15.4, 8.3, 1.5 Hz, 1H), 4.14 (qd, *J* = 7.2, 0.8 Hz, 2H), 3.97 (td, *J* = 8.2, 5.5 Hz, 1H), 3.25 (s, 3H), 2.59 (dd, *J* = 14.9, 8.1 Hz, 1H), 2.42 (dd, *J* = 14.9, 5.5 Hz, 1H), 2.10 – 1.97 (m, 2H), 1.43 – 1.20 (m, 9H), 0.88 (t, *J* = 6.9 Hz, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 171.2, 135.6, 128.7, 79.0, 60.6, 56.2, 41.5, 32.3, 31.5, 28.9, 22.6, 14.4, 14.2. **HRMS** (EI) *m/z*: 228.1717 (*M*⁺); calc. for C₁₃H₂₄O₃: 228.1726. [*α*]_D²³ = +7.504 ± 0.733° (*c* 0.5, CHCl₃). **GC CycloSil-B column** (110 °C), *t_r* = 53.72 min (minor), 55.35 min (major), 94.7 : 5.3 e.r. Absolute stereochemistry was assigned after elaboration to (+)-**6**.

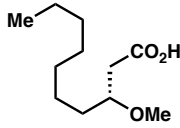
(+)-Ethyl 3-methoxydecanoate ((+)-**6s**)

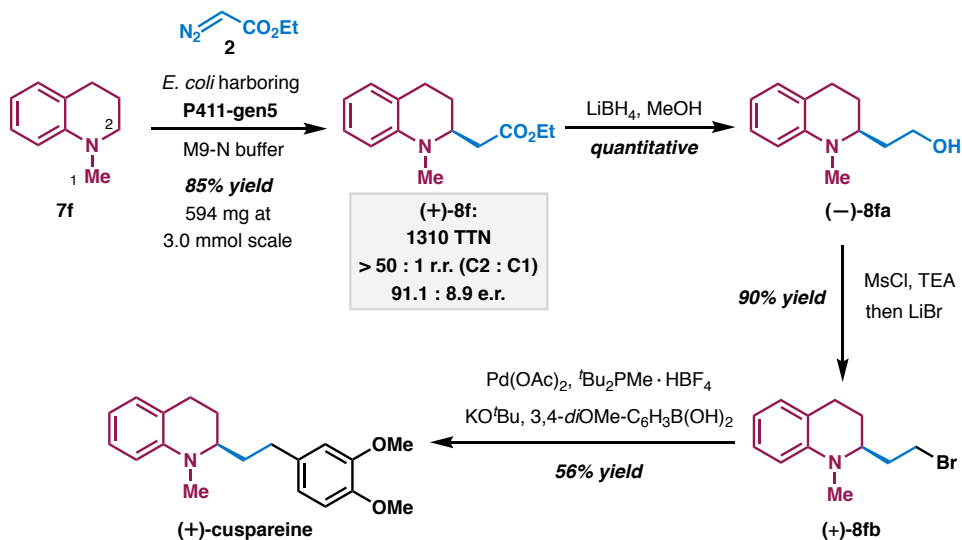
To a solution of (+)-ethyl (*E*)-3-methoxydec-4-enoate ((+)-**5a**, 114.2 mg, 0.5 mmol, 1.0 equiv.) in MeOH (5 mL) was added Pd/C (10% Pd on activated charcoal, 13.3 mg, 2.5 mol%). H₂ was bubbled through the solution for ~30 minutes. The reaction was stirred at room temperature under 1 atm H₂ for 16 hours. The crude product was filtered through a pad of Celite and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded (+)-**6s** (116.9 mg, 0.5 mmol, quantitative yield).

 **¹H NMR** (400 MHz, CDCl₃) δ 4.15 (q, *J* = 7.1 Hz, 2H), 3.63 (ddt, *J* = 7.3, 6.3, 5.5 Hz, 1H), 3.35 (s, 3H), 2.52 (dd, *J* = 15.0, 7.3 Hz, 1H), 2.39 (dd, *J* = 15.0, 5.4 Hz, 1H), 1.57 – 1.41 (m, 2H), 1.40 – 1.20 (m, 13H), 0.88 (t, *J* = 7.1 Hz, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 172.0, 78.0, 60.5, 57.1, 39.7, 34.1, 31.9, 29.8, 29.4, 25.2, 22.8, 14.4, 14.2. **HRMS** (FAB) *m/z*: 231.1965 (*M* + H⁺); calc. for C₁₃H₂₇O₃: 231.1960. [*α*]_D²³ = +4.293 ± 0.136° (*c* 1.0, MeOH). Absolute stereochemistry was assigned after derivatization to (+)-**6**.

(+)-3-Methoxydecanoic acid ((+)-**6**)

To a solution of (+)-ethyl 3-methoxydecanoate ((+)-**6s**, 46.1 mg, 0.2 mmol, 1.0 equiv.) in MeOH (2 mL) was added NaOH (aq., 15%, 2 mL). The reaction mixture was stirred at room temperature for 1 hour and then slowly acidified with HCl (aq., 1 M) at 0 °C until pH 2–3. Extraction by dichloromethane (15 mL × 3), drying over magnesium sulfate, followed by concentration under reduced pressure afforded product **6** (40.8 mg, 0.2 mmol, quantitative yield) without further purification.

 This compound is known in the literature⁴⁶. **¹H NMR** (400 MHz, CDCl₃) δ 3.63 (p, *J* = 6.0 Hz, 1H), 3.39 (s, 3H), 2.55 (dd, *J* = 15.5, 6.9 Hz, 1H), 2.50 (dd, *J* = 15.5, 5.3 Hz, 1H), 1.68 – 1.55 (m, 1H), 1.55 – 1.42 (m, 1H), 1.38 – 1.20 (m, 10H), 0.88 (t, *J* = 7.1 Hz, 3H). **¹³C NMR** (101 MHz, CDCl₃) δ 176.2, 77.7, 57.1, 39.1, 33.6, 31.9, 29.7, 29.4, 25.1, 22.8, 14.2. [*α*]_D²³ = +4.021 ± 1.649° (*c* 1.0, MeOH). The absolute configuration of (+)-**6** was assigned to be (*R*) by comparing the measured optical rotation value with the literature reported value for (*R*)-(+)-**6** (lit. [*α*]_D²⁷ = +3.0°, *c* 0.67, MeOH)⁴⁶. **HRMS** (EI) *m/z*: 202.1598 (*M*⁺); calc. for C₁₁H₂₂O₃: 202.1569.



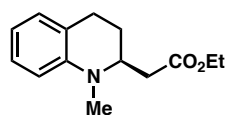
Supplementary Fig. 15. Detailed scheme for the synthesis of (+)-cuspareine.

(+)-Ethyl (*S*)-2-(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)acetate ((+)-8f)

Prepared following **General Procedure D-I** and Workup Procedure D-ii (see **Section IX**).

<i>E. coli</i> suspension in M9-N (variant: P411-gen5, OD ₆₀₀ = 31)				GOX solution ^a	<i>D</i> -glucose in M9-N ^b
Addition 1, volume/ mL	Addition 2, volume/mL	[PC]/μM	n_pro/μmol	volume/mL	volume/mL
275.0	140.0	4.67	1.938	25.0	50.0
Alkane substrate (7f) stock in EtOH			Ethyl diazoacetate stock in EtOH		
stock/M	volume/mL	n_1/mmol	stock/M	volume/mL add. 1; add. 2	n_2/mmol
0.60	5.0	3.0	1.20	2.5; 2.5	6.0
Purification eluent		Product			
1: Hex / (4 : 1 DCM : Et ₂ O)		m[Pdt]/mg	n[Pdt]/mmol	yield	TTN
2: water / MeOH (reverse phase)		594.1 ^c	2.546	85%	1310

^c Regiomer ratio > 50 : 1 for **8f** : **8f'** determined by ¹H NMR.

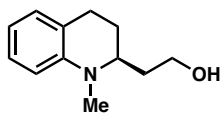


¹H NMR (400 MHz, CDCl₃) δ 7.14 – 7.04 (m, 1H), 6.99 (d, *J* = 7.3 Hz, 1H), 6.63 (td, *J* = 7.3, 1.1 Hz, 1H), 6.55 (d, *J* = 8.2 Hz, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.86 – 3.78 (m, 1H), 2.93 (s, 3H), 2.91 – 2.79 (m, 1H), 2.71 (ddd, *J* = 16.6, 5.5, 2.8 Hz, 1H), 2.60 (ddd, *J* = 14.7, 5.4, 0.8 Hz, 1H), 2.39 (dd, *J* = 14.7, 8.6 Hz, 1H), 2.07 – 1.95 (m, 1H), 1.89 (ddt, *J* = 13.4, 5.8, 2.9 Hz, 1H), 1.27 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 172.3, 144.8, 129.0, 127.3, 121.6, 116.2, 111.0, 60.7, 56.1, 37.8, 36.6, 25.5, 23.3, 14.4. HRMS (FAB) *m/z*: 233.1405 (M⁺); calc. for C₁₄H₁₉NO₂: 233.1416. With variant **P411-gen5**: [α]_D²³ = +9.440 ± 0.292° (*c* 0.5, CHCl₃). With variant **P411-gen5**: SFC Chiralcel OB-H column (supercritical CO₂/isopropanol = 97:3, 2.5 mL/min, 40 °C), *t*_r = 6.44 min (minor), 7.25 min (major), 91.1 : 8.9 e.r. Absolute stereochemistry was assigned after elaboration

to (+)-cuspareine.

(-)-2-(1-Methyl-1,2,3,4-tetrahydroquinolin-2-yl)ethan-1-ol ((-)-8fa)

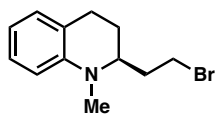
To a solution of (+)-ethyl 2-(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)acetate ((+)-8f, 233.3 mg, 1.0 mmol, 1.0 equiv.) and MeOH (60.8 μ L, 1.5 mmol, 1.5 equiv.) in dry diethyl ether (20 mL) was added LiBH₄ (2 M in THF, 1.0 mL, 2.0 mmol, 2.0 equiv.) dropwise. The reaction mixture was heated to reflux (~45 °C) for 4 hours (monitored by TLC). Upon completion, the reaction was quenched by ethyl acetate (2 mL) and stirred for 30 min at room temperature, quenched by MeOH (2 mL) and stirred for another 20 min at 0 °C, and finally quenched by NH₄Cl (sat. aq., 5 mL) at 0 °C. The crude product was then extracted by diethyl ether (30 mL \times 3). The combined organic layer was washed by brine (30 mL), dried over sodium sulfate and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded **8fa** (192.1 mg, 1.0 mmol, quantitative yield).



This compound is known in the literature⁴⁷. ¹H NMR (400 MHz, CDCl₃) δ 7.12 – 7.06 (m, 1H), 7.01 – 6.96 (m, 1H), 6.62 (td, J = 7.3, 1.2 Hz, 1H), 6.59 (dd, J = 8.2, 1.1 Hz, 1H), 3.82 – 3.71 (m, 2H), 3.51 – 3.44 (m, 1H), 2.97 (s, 3H), 2.88 – 2.76 (m, 1H), 2.70 (ddd, J = 16.4, 5.3, 3.4 Hz, 1H), 2.00 – 1.81 (m, 3H), 1.72 – 1.61 (m, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 145.4, 129.0, 127.3, 122.2, 116.2, 112.0, 60.6, 56.2, 39.1, 34.6, 24.8, 23.8. $[\alpha]_D^{23}$ = $-28.096 \pm 2.416^\circ$ (c 0.5, CHCl₃). Absolute stereochemistry was assigned after derivatization to (+)-cuspareine.

(+)-2-(2-Bromoethyl)-1-methyl-1,2,3,4-tetrahydroquinoline ((+)-8fb)

To a solution of (-)-2-(1-Methyl-1,2,3,4-tetrahydroquinolin-2-yl)ethan-1-ol ((-)-8fa, 114.8 mg, 0.6 mmol, 1.0 equiv.) and triethylamine (133.8 μ L, 0.96 mmol, 1.6 equiv.) in dry THF (12 mL) at 0 °C was added methanesulfonyl chloride (MsCl, 70 μ L, 1.5 equiv.). The reaction mixture was warmed to room temperature over 30 min and stirred for additional 30 min. Upon completion, the reaction was quenched by NaHCO₃ (sat. aq., 10 mL) and H₂O (10 mL). The mesylated product was then extracted by diethyl ether (30 mL \times 3). The combined organic layer was washed by brine (30 mL), dried over sodium sulfate and concentrated under reduced pressure. The mesylated product was then dissolved in DMF (6 mL) and LiBr (259.5 mg, 3.0 mmol, 5.0 equiv.) was added. The reaction was heated to 70 °C and stirred for 2 hours. Upon completion, the reaction was quenched by H₂O (30 mL). The crude product was then extracted by diethyl ether (30 mL \times 3). The combined organic layer was washed by H₂O (30 mL) and brine (30 mL), dried over sodium sulfate and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded **8fb** (136.8 mg, 0.54 mmol, 90% yield).

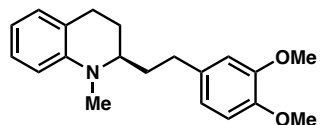


¹H NMR (400 MHz, CDCl₃) δ 7.12 – 7.06 (m, 1H), 7.01 – 6.95 (m, 1H), 6.61 (td, J = 7.3, 1.2 Hz, 1H), 6.56 (dd, J = 8.2, 1.1 Hz, 1H), 3.58 – 3.47 (m, 2H), 3.43 (ddd, J = 10.1, 7.7, 6.3 Hz, 1H), 2.99 (s, 3H), 2.84 – 2.66 (m, 2H), 2.22 – 2.11 (m, 1H), 2.01 – 1.90 (m, 2H), 1.90 – 1.81 (m, 1H). ¹³C NMR (101 MHz, CDCl₃) δ 145.1, 129.0, 127.4, 121.5, 116.0, 111.2, 57.0, 38.7, 34.9, 31.0, 24.3, 23.6. HRMS (FAB) m/z : 254.0548 ($M + H^+$); calc. for C₁₂H₁₇⁷⁹BrN: 254.0544. $[\alpha]_D^{23}$ = $+33.638 \pm 2.022^\circ$ (c 0.5, CHCl₃). Absolute stereochemistry was assigned after derivatization to (+)-cuspareine.

(+)-Cuspareine

(+)-Cuspareine was synthesized through Suzuki-Miyaura cross-coupling between an alkyl bromide and an aryl boronic acid. The reaction conditions for this cross-coupling are derived from those described by Fu *et al.*⁴⁸.

To a 50-mL resealable Schlenk tube were added Pd(OAc)₂ (4.04 mg, 0.018 mmol, 15 mol%), di-*tert*-butyl(methyl)phosphonium tetrafluoroborate (*t*Bu₂PMe·HBF₄, 8.93 mg, 0.036 mmol, 30 mol%), KO*t*Bu (40.4 mg, 0.36 mmol, 3.0 equiv.) and *t*Amyl-OH (0.6 mL). The tube was charged with Ar and sealed. The mixture was stirred at 60 °C for 20 min until the color of mixture turned pale yellow. After the mixture was cooled to room temperature, (3,4-dimethoxyphenyl)boronic acid (54.6 mg, 0.30 mmol, 2.5 equiv.) and a solution of (+)-2-(2-bromoethyl)-1-methyl-1,2,3,4-tetrahydroquinoline ((+)-**8fb**, 30.5 mg, 0.12 mmol, 1.0 equiv.) in *t*Amyl-OH (0.6 mL) were added to the tube. Another portion of *t*Amyl-OH (0.8 mL) was used to wash the solution residue of (+)-**8fb** and then transferred to the tube. The tube was charged with Ar and sealed again. After the reaction mixture was stirred at 60 °C for 15 hours, it was then cooled to room temperature, diluted with diethyl ether (6 mL), filtrated through a pad of Celite, washed by diethyl ether (30 mL) and concentrated under reduced pressure. Purification by silica column chromatography with hexanes / ethyl acetate afforded (+)-cuspareine (20.8 mg, 0.067 mmol, 56% yield).



Spectral data is in agreement with literature report⁴⁹. **¹H NMR** (400 MHz, CDCl₃) δ 7.09 (td, *J* = 7.6, 1.4 Hz, 1H), 6.99 (d, *J* = 7.0 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 6.75 – 6.70 (m, 2H), 6.60 (td, *J* = 7.3, 1.1 Hz, 1H), 6.54 (d, *J* = 8.2 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.33 – 3.26 (m, 1H), 2.92 (s, 3H), 2.91 – 2.80 (m, 1H), 2.74 – 2.63 (m, 2H), 2.54 (ddd, *J* = 13.9, 10.1, 6.4 Hz, 1H), 2.01 – 1.87 (m, 3H), 1.74 (dddd, *J* = 13.6, 10.1, 8.8, 5.4 Hz, 1H). **¹³C NMR** (101 MHz, CDCl₃) δ 149.0, 147.3, 145.4, 134.8, 128.8, 127.2, 121.8, 120.2, 115.5, 111.7, 111.4, 110.7, 58.5, 56.1, 56.0, 38.2, 33.2, 32.1, 24.5, 23.7. [α]_D²³ = +23.404 ± 0.723° (*c* 0.5, CHCl₃). The absolute configuration of (+)-cuspareine was assigned to be (*R*) by comparing the measured optical rotation value with the literature reported value for (*R*)-(+)-cuspareine (lit. [α]_D²⁷ = +23.516°, *c* 0.8, CHCl₃)⁴⁹.

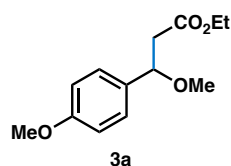
XI. Determination of enantioselectivity

Enantioselectivity of enzymatic C–H alkylation products were determined by chiral HPLC, chiral SFC, or chiral GC analysis. Representative traces, and their conditions, are shown below.

The absolute configuration of P411-CHF synthesized **3i** was assigned to be (*S*) by comparing the chiral HPLC separation of *rac*-**3i** and P411-CHF synthesized **3i** with that reported in the literature⁵⁰. The absolute configuration was further confirmed by comparing the optical rotation values for P411-CHF synthesized **3i** and (*S*)-(+)-**3i** as reported in the literature⁵⁰. The absolute configurations of **3a–3h** and **3j–3m** were inferred by analogy, assuming the selectivity with P411-CHF remains the same in the enzymatic syntheses of those compounds.

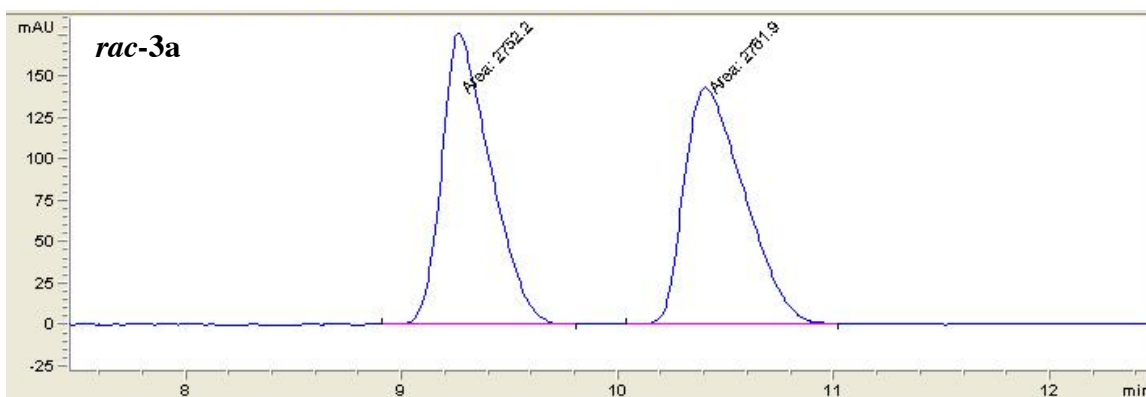
The absolute configuration of P411-CHF synthesized (+)-**5a** was determined to be (*S*) after elaboration to (+)-**6** (see **Section X** for more details). The absolute configurations of **5b–5e** were inferred by analogy, assuming the selectivity with P411-CHF remains the same in the enzymatic syntheses of those compounds.

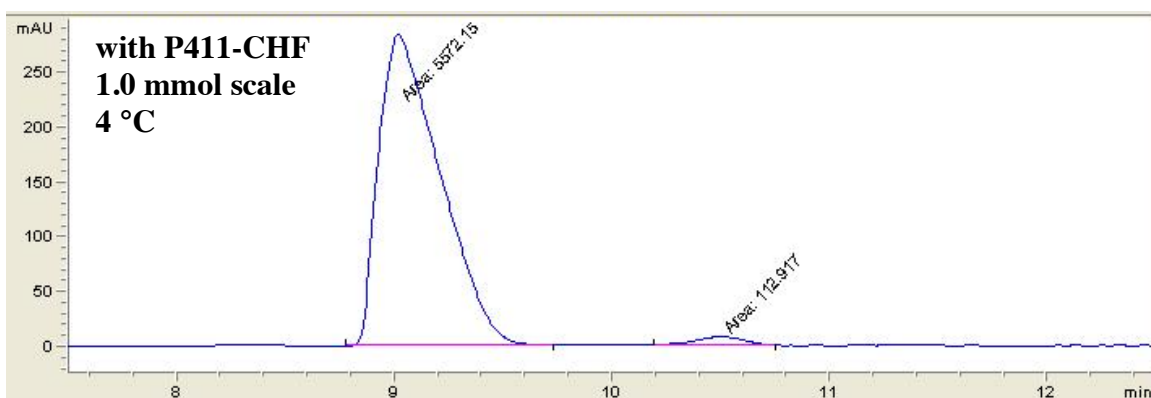
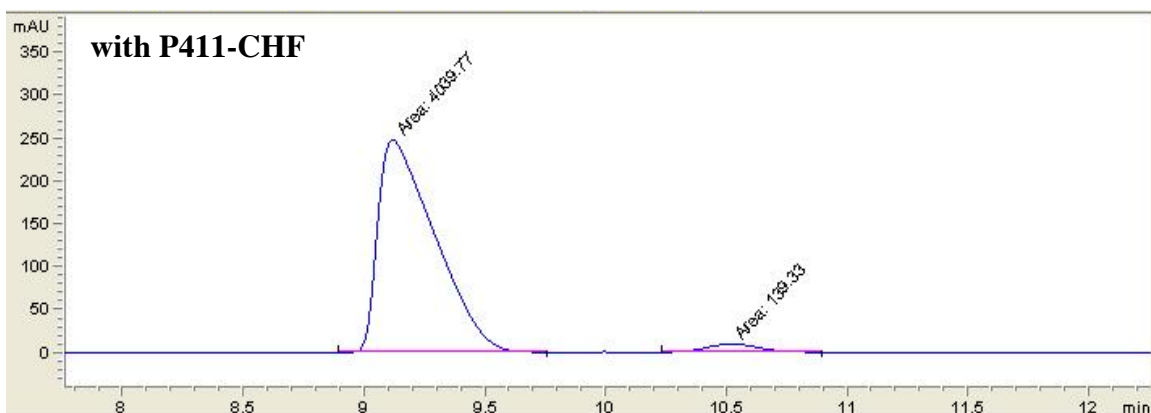
The absolute configuration of P411-gen5 synthesized (+)-**8f** was determined to be (*S*) after elaboration to (+)-cuspamine (see **Section X** for more details). As P411-CHF shows the opposite selectivity for the synthesis of (–)-**8f**, P411-CHF produces (*R*)-**8f** in excess.



Ethyl 3-methoxy-3-(4-methoxyphenyl)propanoate (3a)

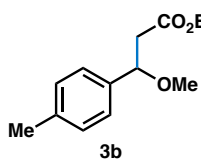
SFC (Chiralpak AD-H column): 3% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 235 nm.





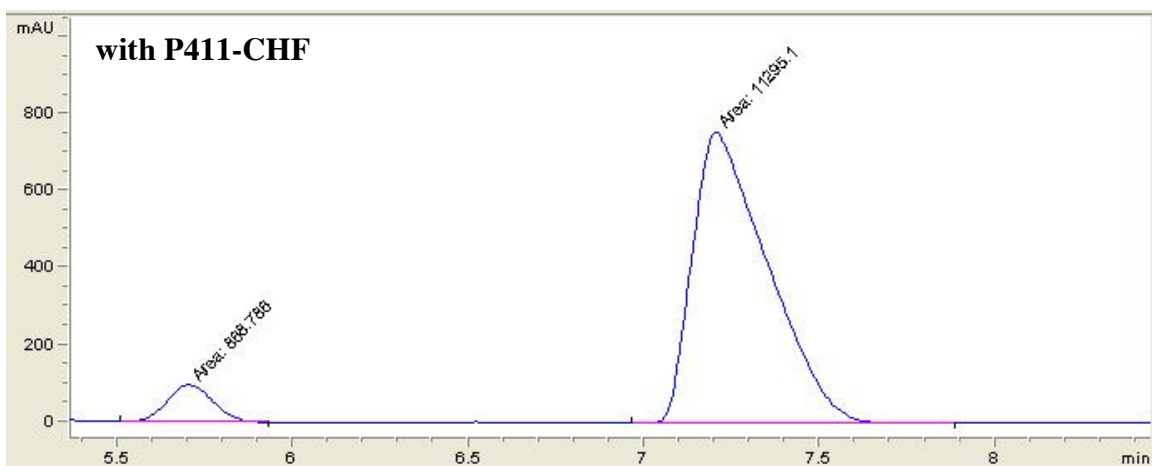
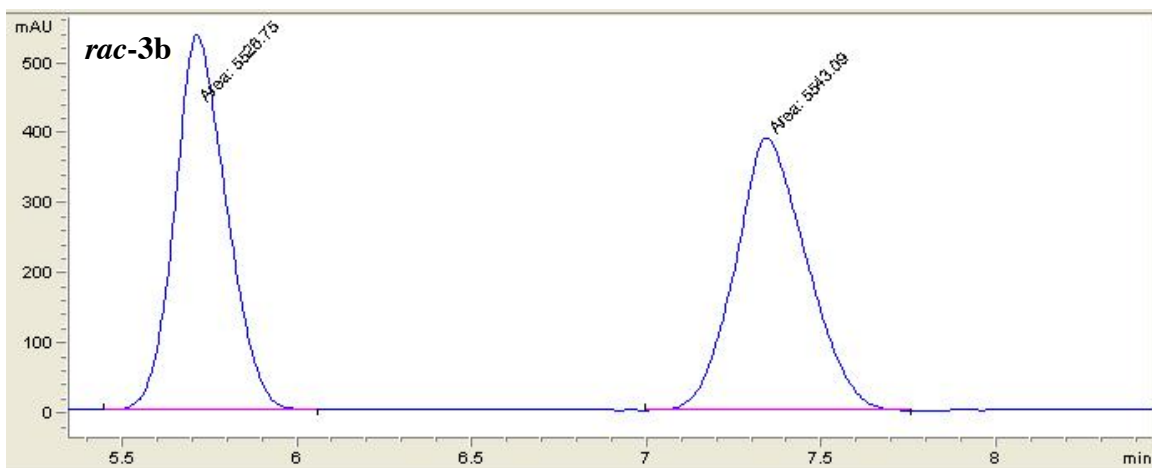
<i>rac-3a</i>			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
9.262	2752.2	49.91	9.119	4039.8	96.67
10.404	2761.9	50.09	10.524	139.3	3.33
Total	5514.1	100.00	Total	4179.1	100.00

with P411-CHF, 1.0 mmol scale, 4 °C		
Retention Time (min)	Area (mAU*s)	Area %
9.016	5572.2	98.01
10.501	112.9	1.99
Total	5685.1	100.00

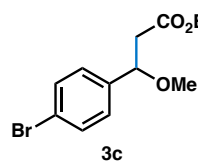


Ethyl 3-methoxy-3-(*p*-tolyl)propanoate (3b)

SFC (Chiralpak AD-H column): 2% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 210 nm.

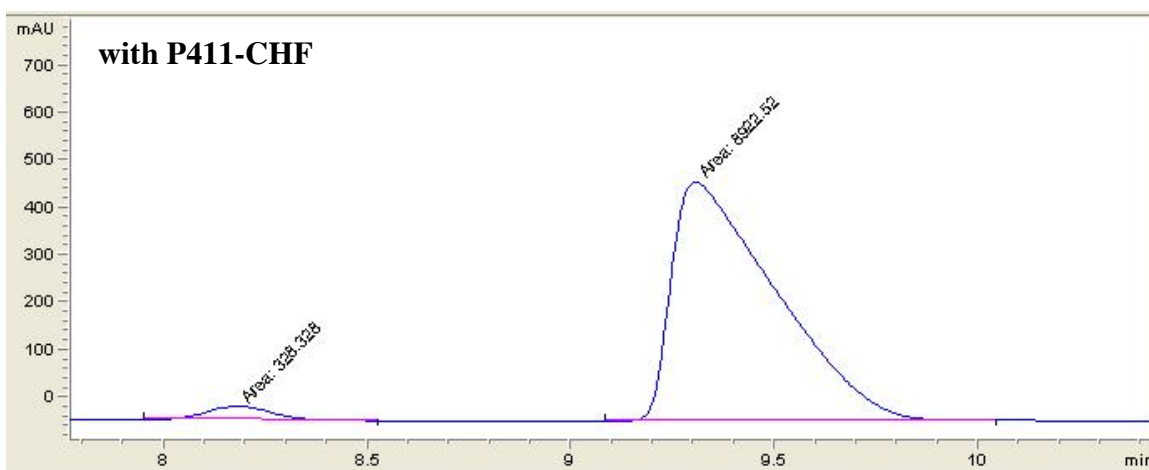
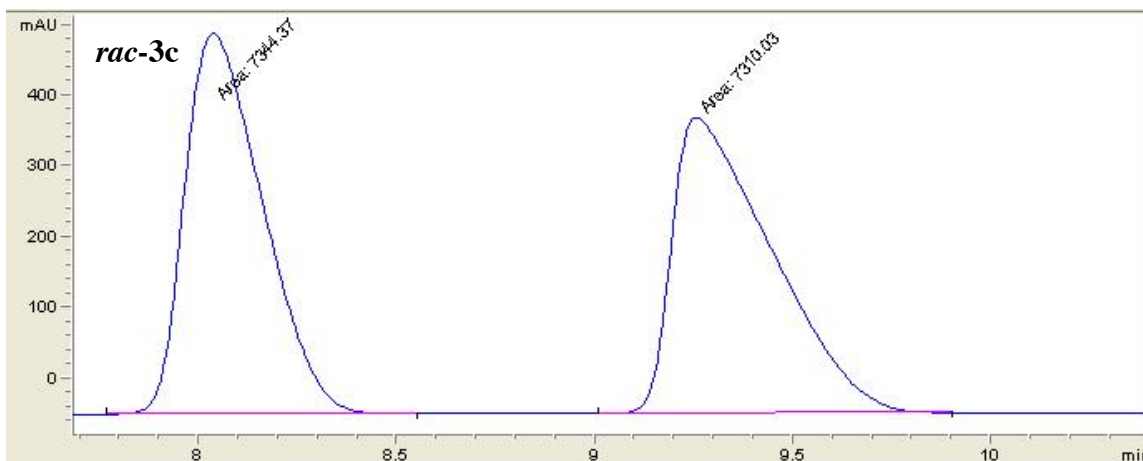


rac-3b			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
5.712	5526.8	49.93	5.705	868.8	7.14
7.342	5543.1	50.07	7.205	11295.1	92.86
Total	11069.9	100.00	Total	12163.9	100.00

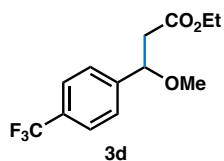


Ethyl 3-(4-bromophenyl)-3-methoxypropanoate (**3c**)

SFC (Chiralpak AD-H column): 3% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 210 nm.

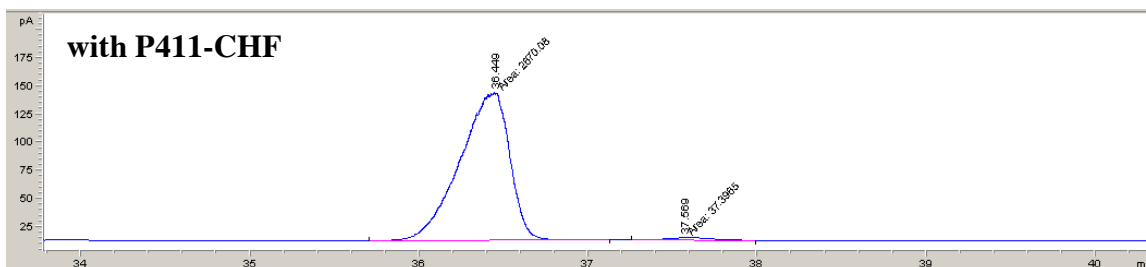
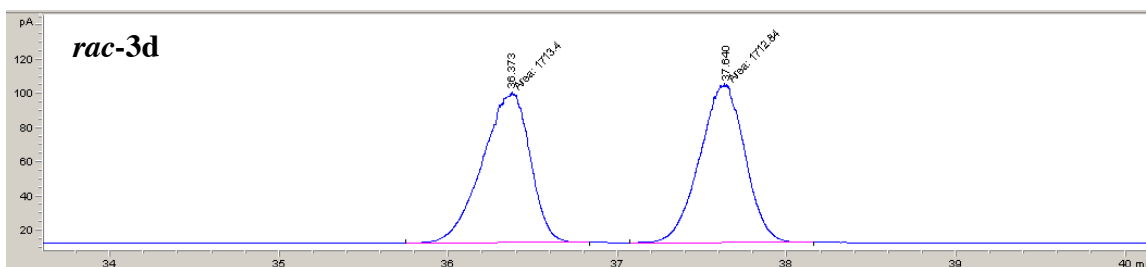


rac-3c			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
8.037	7344.4	50.12	8.181	328.3	3.55
9.256	7310.0	49.88	9.306	8922.5	96.45
Total	14654.4	100.00	Total	9250.8	100.00

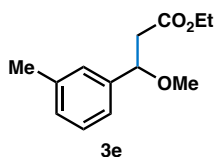


Ethyl 3-methoxy-3-(4-(trifluoromethyl)phenyl)propanoate (3d)

GC (Chiraldex G-TA column): 110 °C

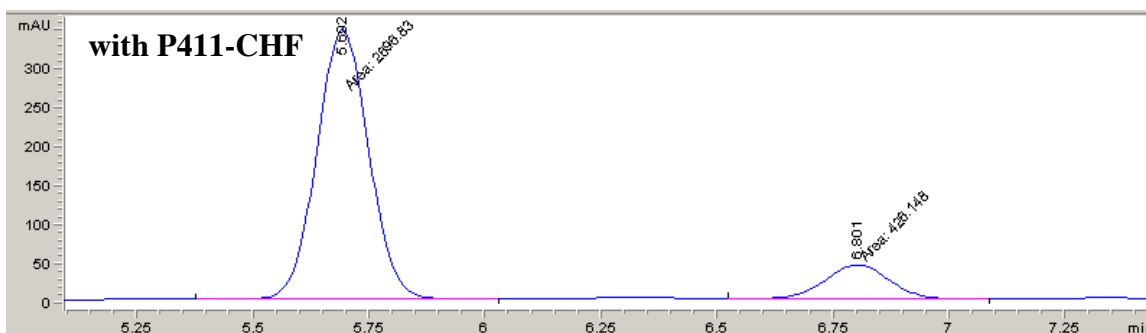
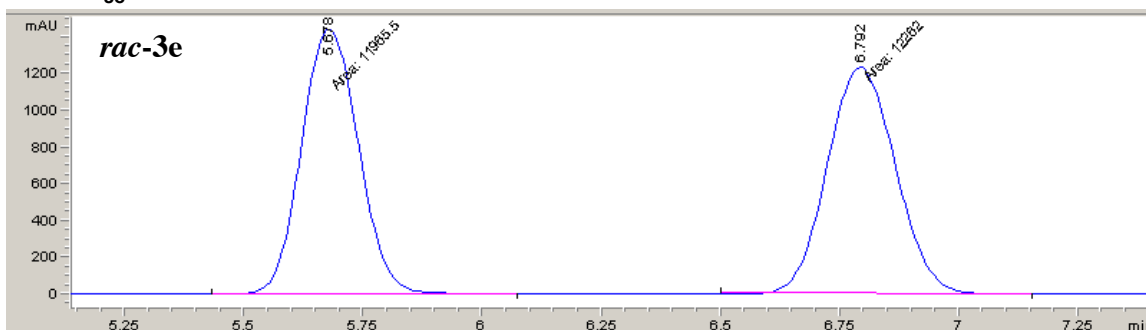


rac-3d			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
36.373	1713.4	50.01	36.449	2670.1	98.62
37.640	1712.8	49.99	37.569	37.4	1.38
Total	3426.2	100.00	Total	2707.5	100.00

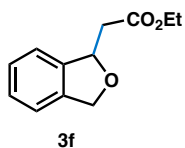


Ethyl 3-methoxy-3-(*m*-tolyl)propanoate (3e)

HPLC (Chiralpak IC column): 4% *i*-PrOH in hexane, 1.5 mL/min, 23 °C, 210 nm

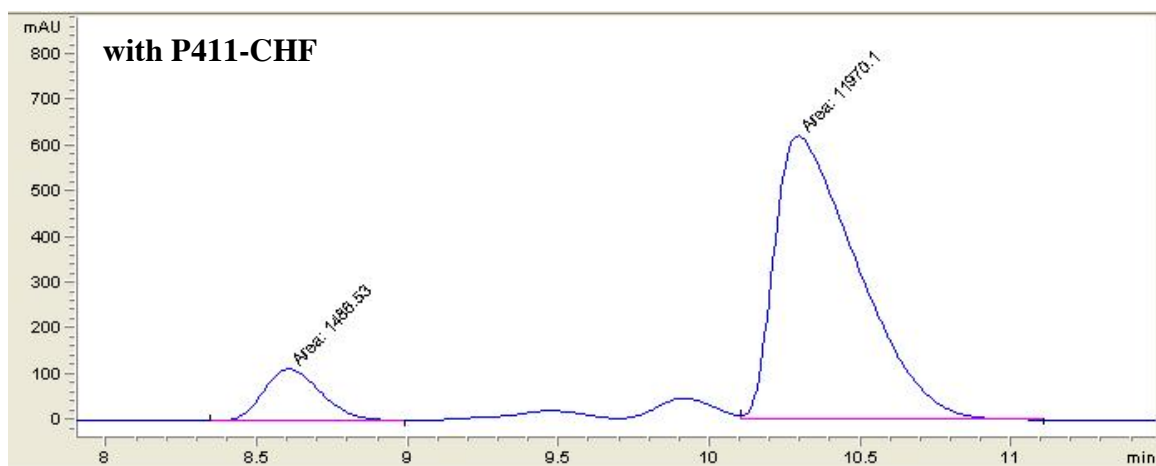
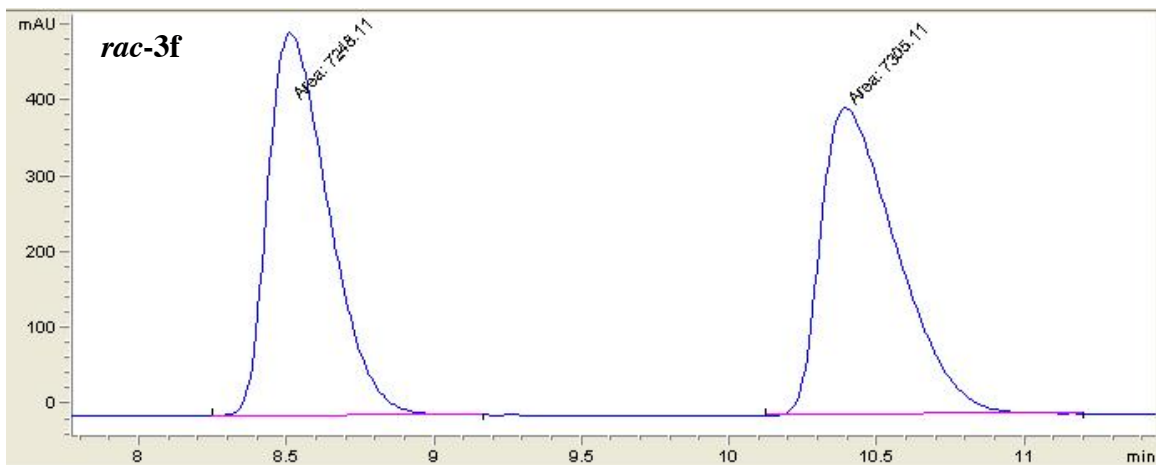


rac-3e			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
5.678	11965.5	49.39	5.692	2696.8	86.35
6.792	12262.0	50.61	6.801	426.1	13.65
Total	24227.5	100.00	Total	3122.9	100.00

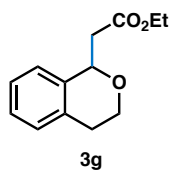


Ethyl 2-(1,3-dihydroisobenzofuran-1-yl)acetate (3f)

SFC (Chiralpak AD-H column): 3% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 210 nm

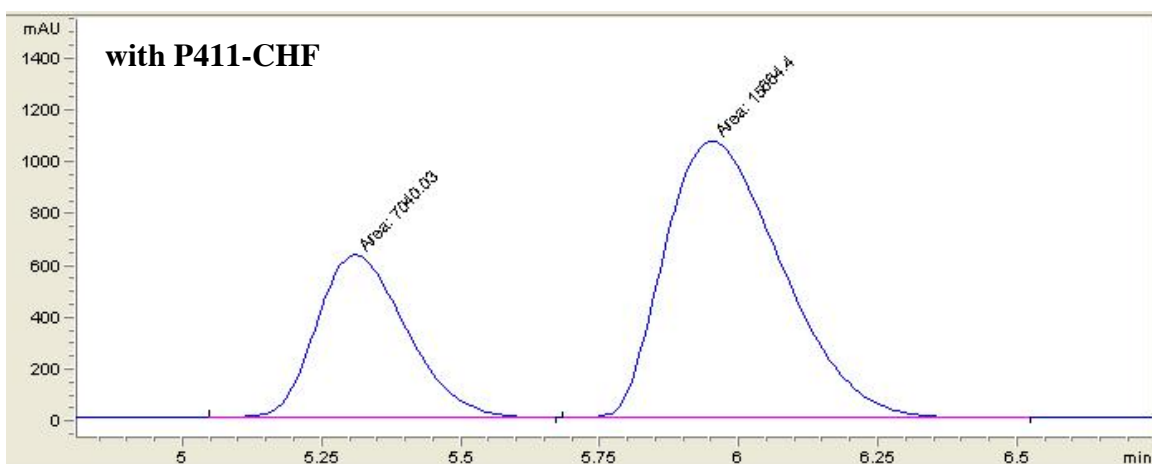
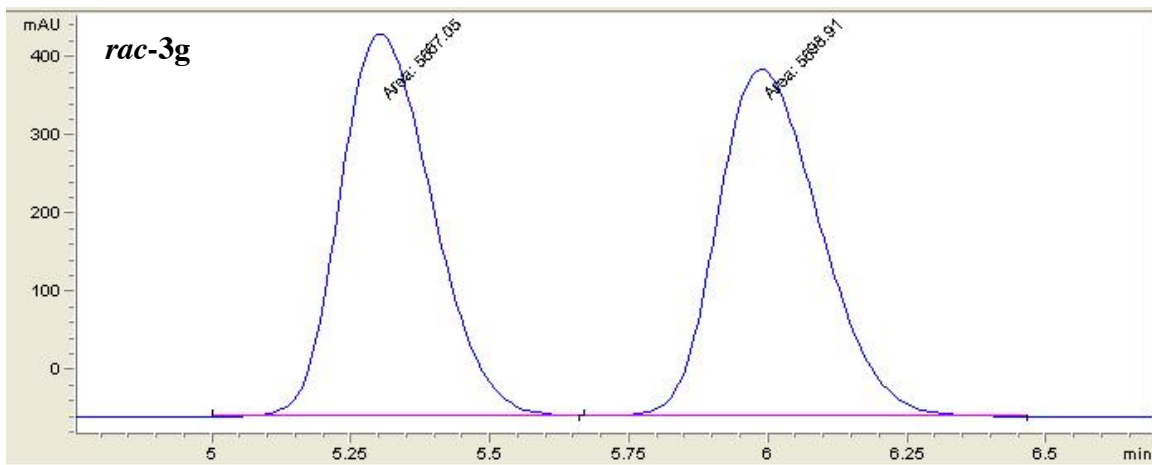


rac-3f			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
8.511	7248.1	49.80	8.604	1486.5	11.05
10.392	7305.1	50.20	10.294	11970.1	88.95
Total	14553.2	100.00	Total	13456.6	100.00

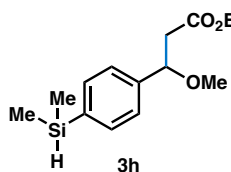


Ethyl 2-(isochroman-1-yl)acetate (3g)

SFC (Chiralcel OJ-H column): 0.2% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 210 nm

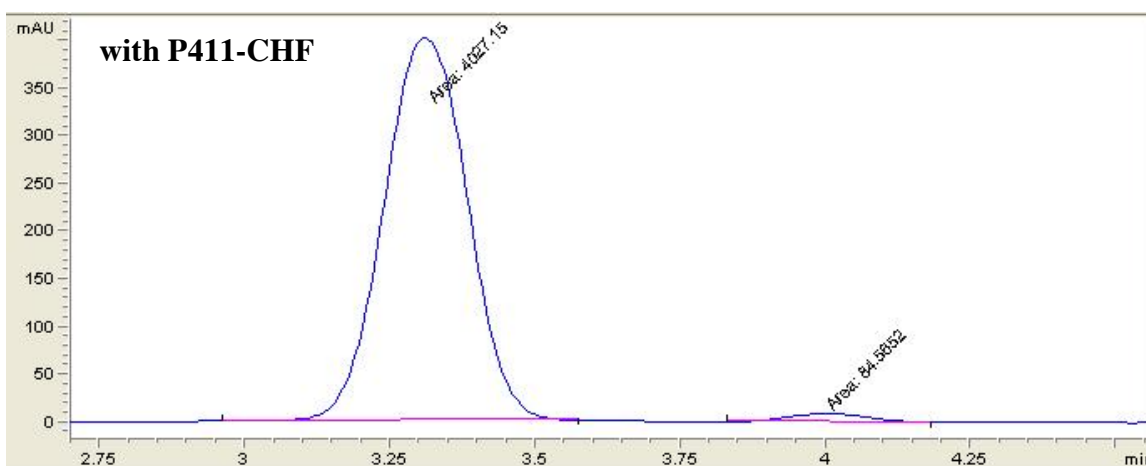
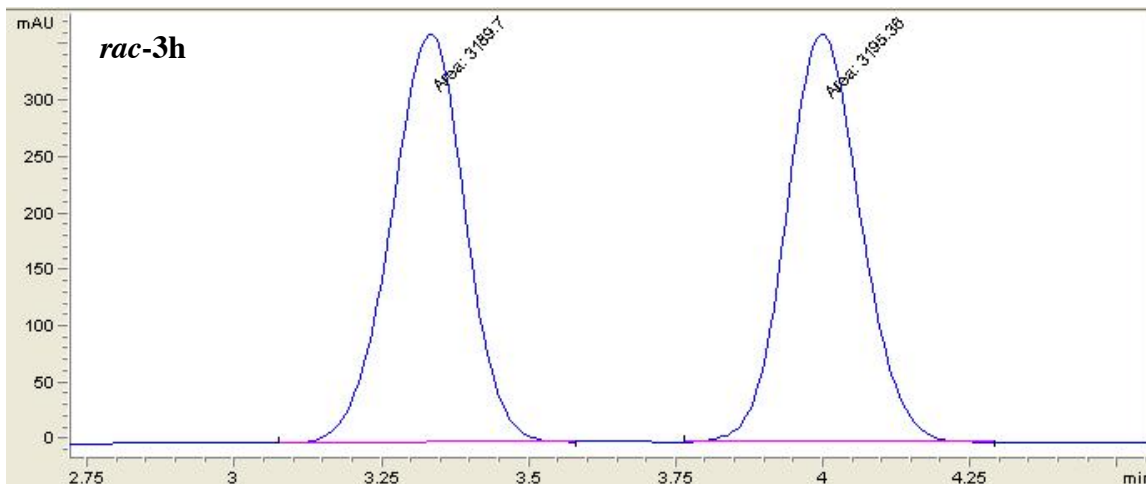


rac-3g			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
5.301	5667.0	49.86	5.309	7040.0	31.01
5.987	5698.9	50.14	5.951	15664.4	68.99
Total	11365.9	100.00	Total	22704.4	100.00

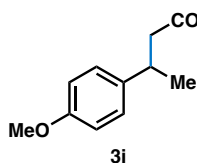


Ethyl 3-(4-(dimethylsilyl)phenyl)-3-methoxypropanoate (**3h**)

SFC (Chiralcel OD-H column): 2% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 210 nm

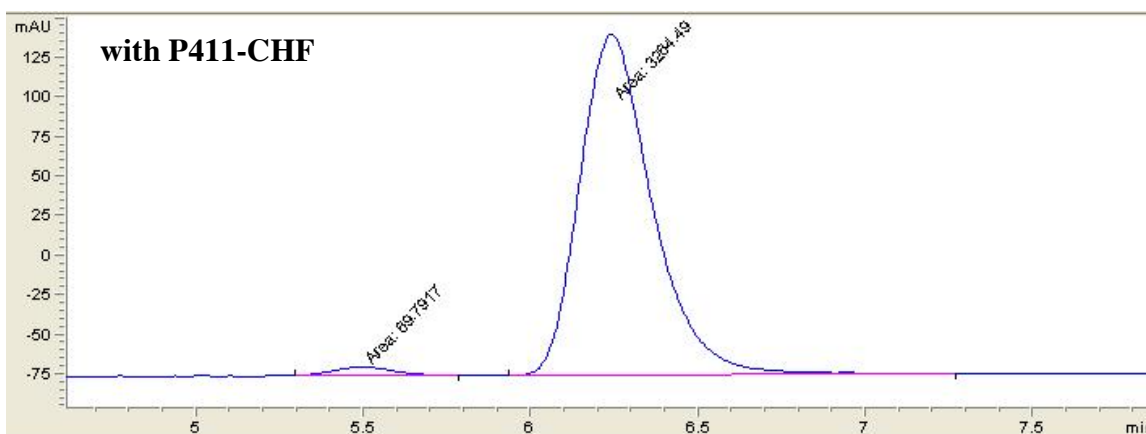
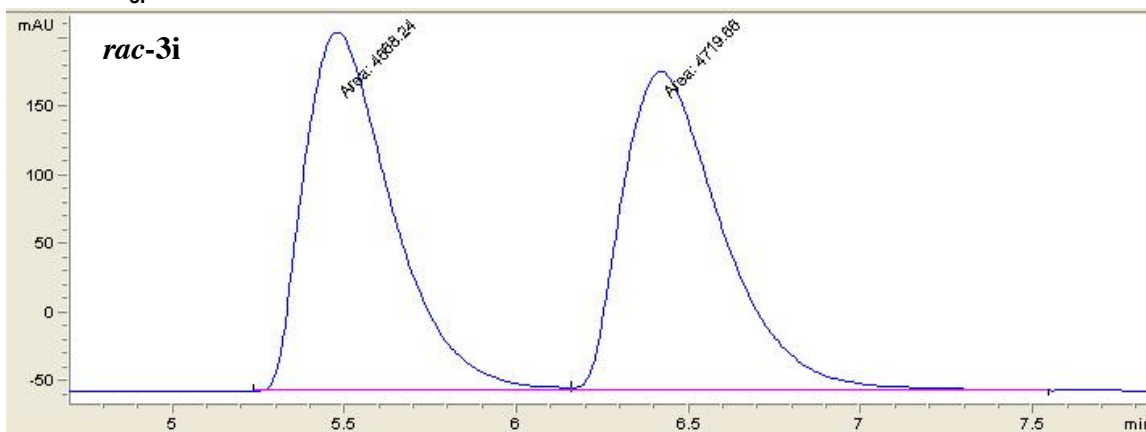


rac-3h			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
3.332	3189.7	49.96	3.309	4027.1	97.94
3.998	3195.4	50.04	3.997	84.6	2.06
Total	6385.1	100.00	Total	4111.7	100.00



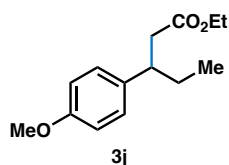
Ethyl 3-(4-methoxyphenyl)butanoate (**3i**)

SFC (Chiralcel OB-H column): supercritical CO₂, 2.5 mL/min, 40 °C, 210 nm.



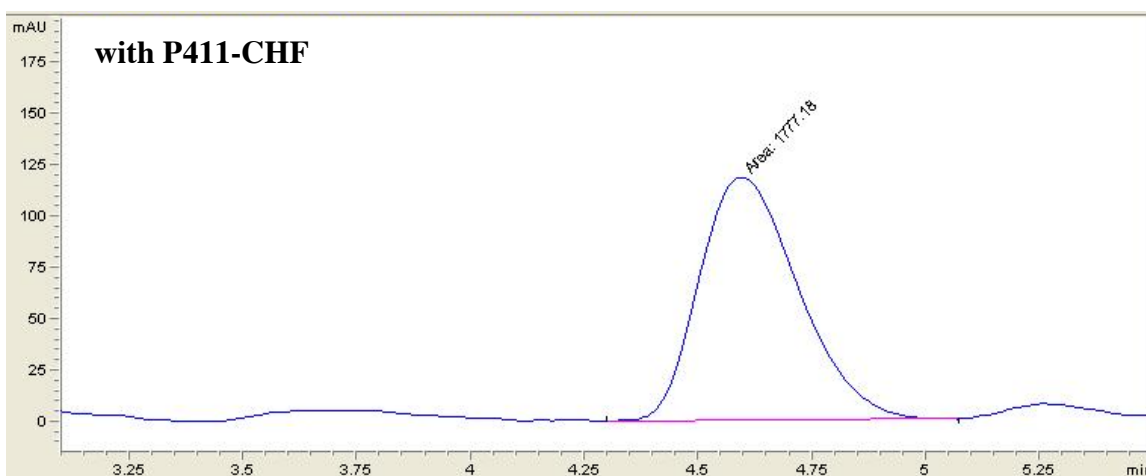
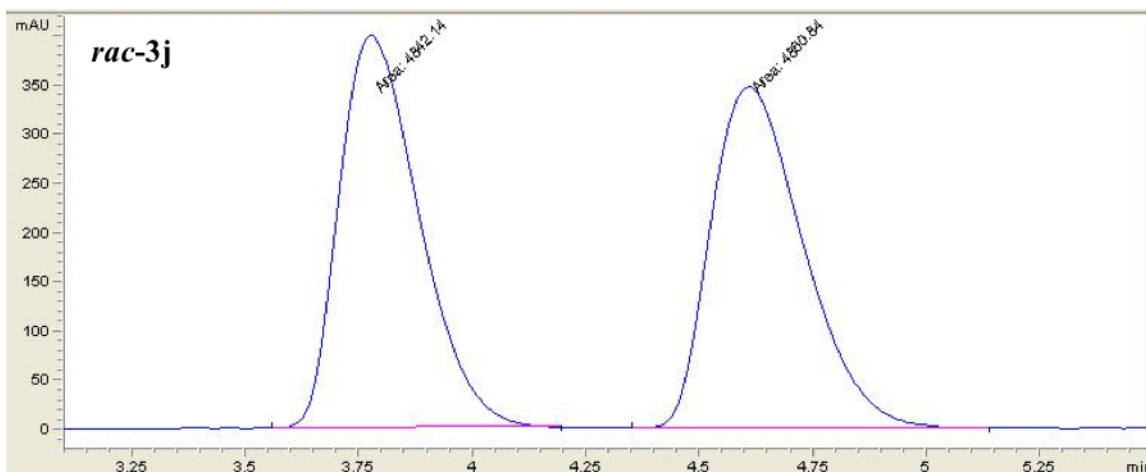
<i>rac</i>-3i			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
5.478	4668.2	49.73	5.496	69.8	2.09
6.417	4719.7	50.27	6.240	3264.5	97.91
Total	9387.9	100.00	Total	3334.3	100.00

The absolute configuration of P411-CHF synthesized **3i** was determined to be (*S*) by comparing the chiral HPLC separation (Chiralcel OJ-H column, 1% *i*-PrOH in hexanes, traces not shown) of *rac*-**3i** and P411-CHF synthesized **3i** with that reported in the literature⁵⁰. The absolute configuration was further confirmed by comparing the optical rotation value for P411-CHF synthesized **3i**, $[\alpha]_D^{23} = +26.3^\circ$ (*c* 0.5, CHCl₃, see **Section XI**), and (*S*)-(+)-**3i** as reported in the literature ($[\alpha]_D^{23} = +26.2^\circ$, *c* 0.59, CHCl₃)⁵⁰.

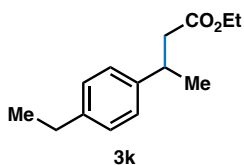


Ethyl 3-(4-methoxyphenyl)pentanoate (**3j**)

SFC (Chiralcel OB-H column): 1% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 210 nm.

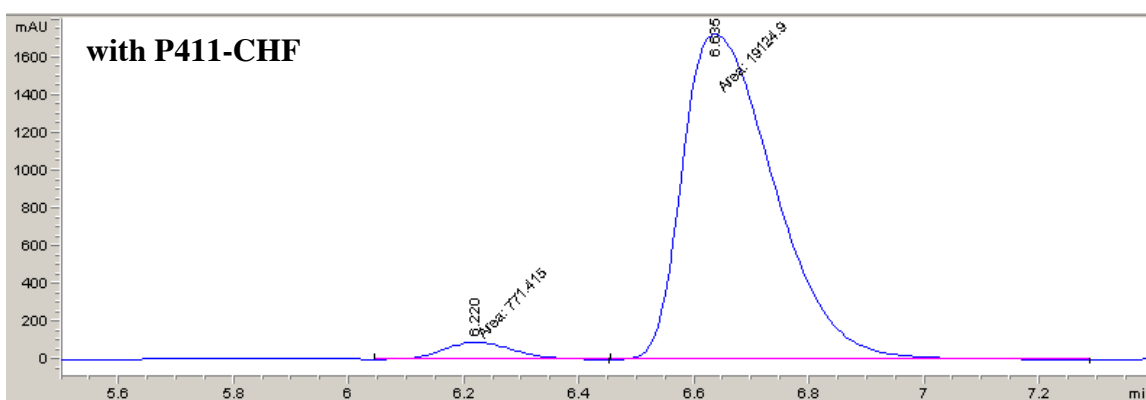
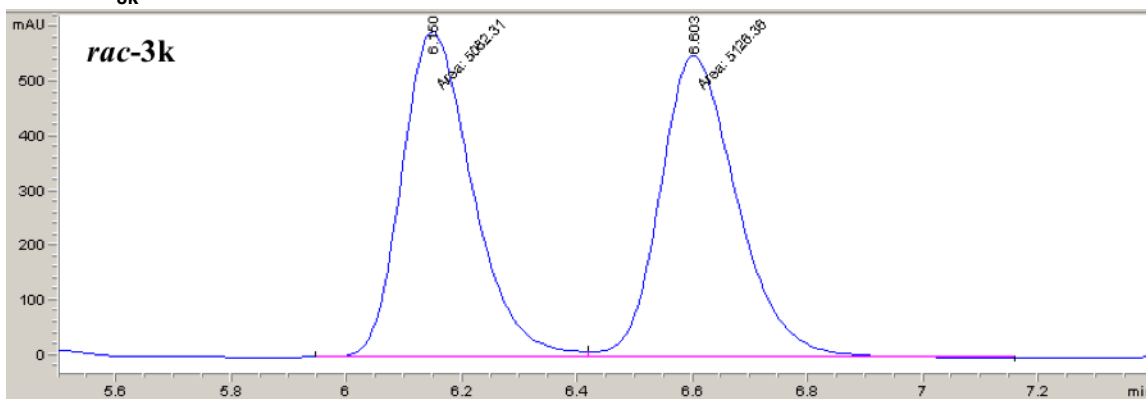


rac-3j			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
3.778	4842.1	49.90	3.788	n. d.	-
4.609	4860.8	50.10	4.595	1777.2	100.00
Total	9702.9	100.00	Total	1777.2	100.00

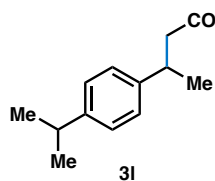


Ethyl 3-(4-ethylphenyl)butanoate (3k)

HPLC (Chiralcel OD-H column): 3% *i*-PrOH in hexane, 1.0 mL/min, 32 °C, 210 nm.

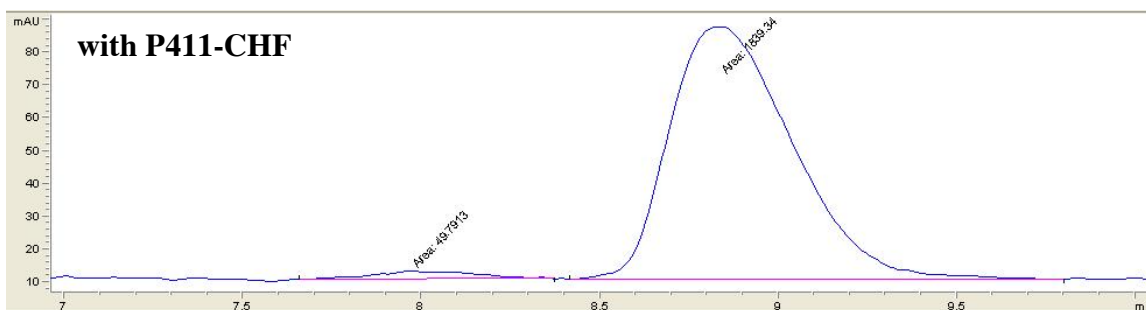
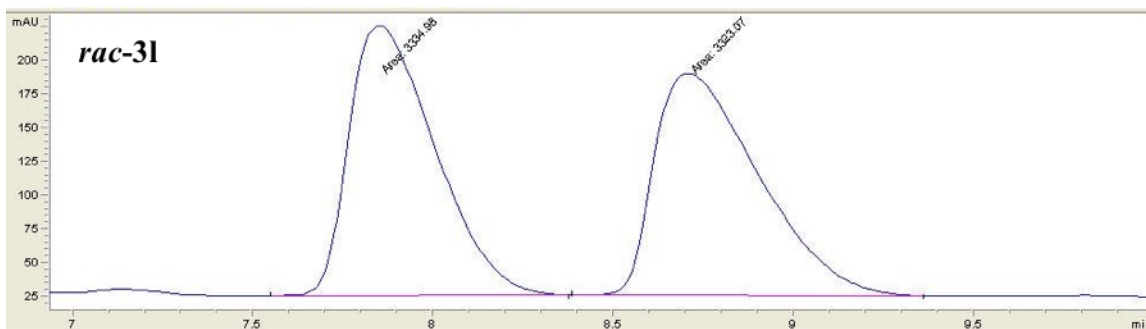


rac-3k			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
6.150	5082.3	49.78	6.220	771.4	3.88
6.603	5126.4	50.22	6.635	19124.9	96.12
Total	10208.7	100.00	Total	19896.3	100.00

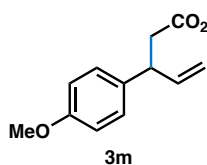


Ethyl 3-(4-isopropylphenyl)butanoate (**31**)

SFC (Chiralpak IC column): 0.5% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 210 nm.

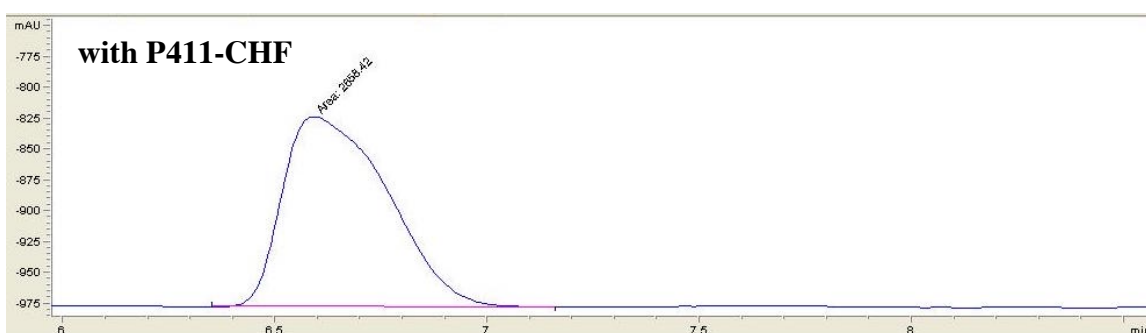
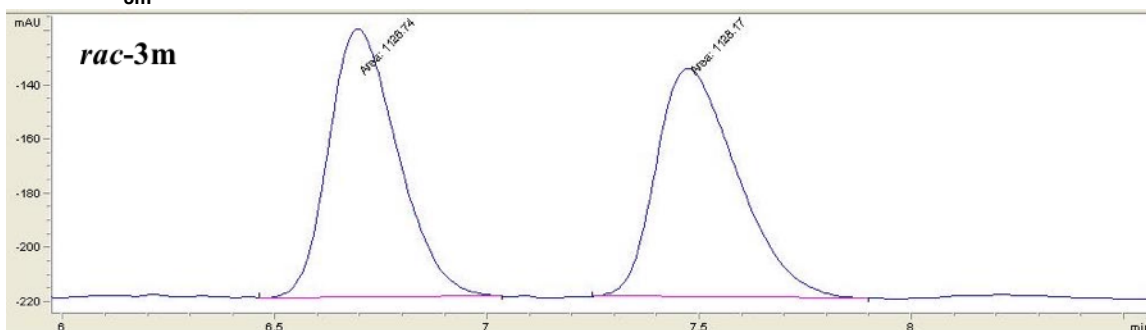


rac-31			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
7.850	3335.0	50.09	7.970	49.8	2.64
8.707	3323.1	49.91	8.836	1839.3	97.36
Total	6658.1	100.00	Total	1889.1	100.00

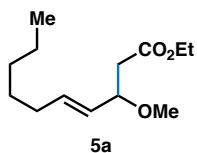


Ethyl 3-(4-methoxyphenyl)pent-4-enoate (3m)

SFC (Chiralpak AD-H column): 1% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 210 nm.

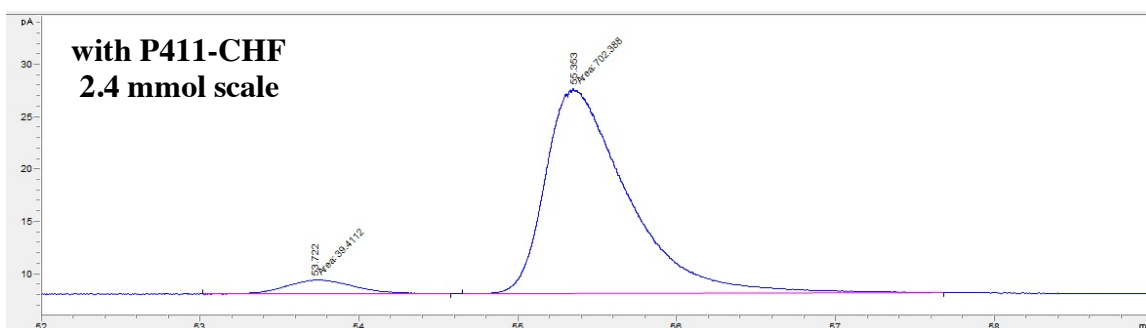
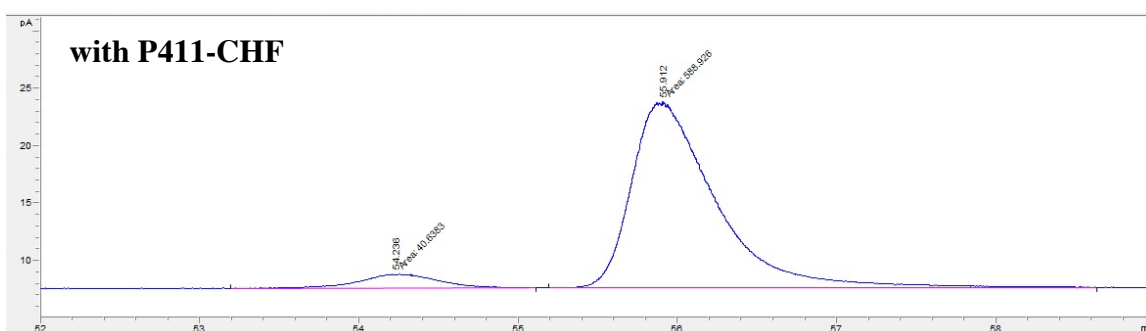
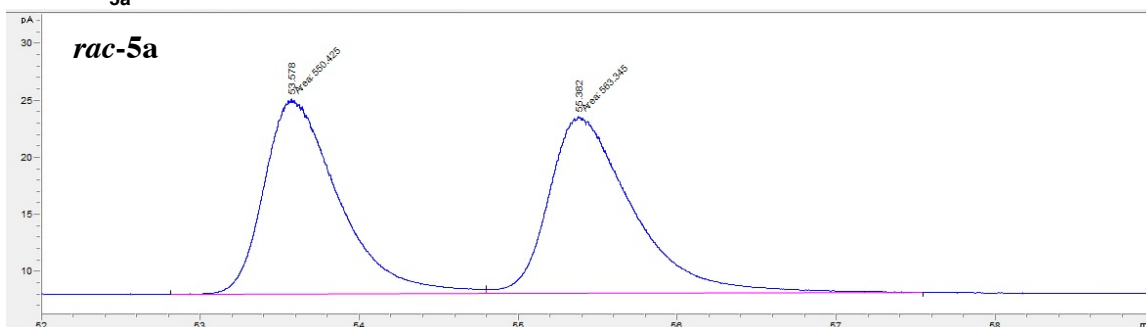


rac-3m			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
6.694	1126.7	49.97	6.591	2658.4	100.00
7.472	1128.2	50.03	7.472	n. d.	-
Total	2254.9	100.00	Total	2658.4	100.00



Ethyl (*E*)-3-methoxydec-4-enoate (5a)

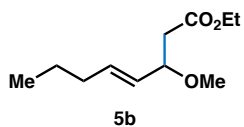
GC (CycloSil-B column): 110 °C



rac-5a			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
53.578	550.4	49.42	54.236	40.6	6.45
55.382	563.3	50.58	55.912	588.9	93.55
Total	1113.7	100.00	Total	629.5	100.00

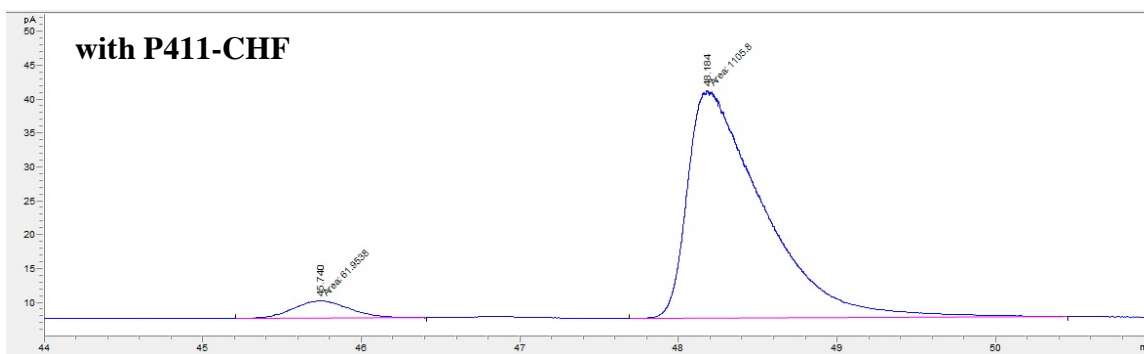
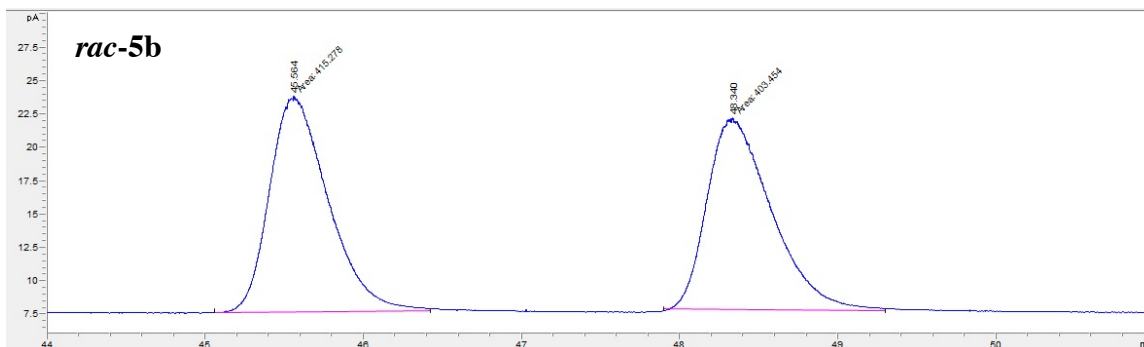
with P411-CHF, 2.4 mmol scale		
Retention Time (min)	Area (mAU*s)	Area %
53.722	39.4	5.31
55.353	702.4	94.69
Total	741.8	100.00

The absolute configuration of P411-CHF synthesized **5a** was determined to be (*S*) after derivatization to (+)-**6** (see **Section X** for more details).

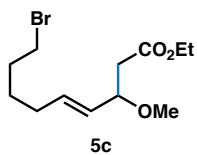


Ethyl (*E*)-3-methoxyoct-4-enoate (5b)

GC (CycloSil-B column): 90 °C

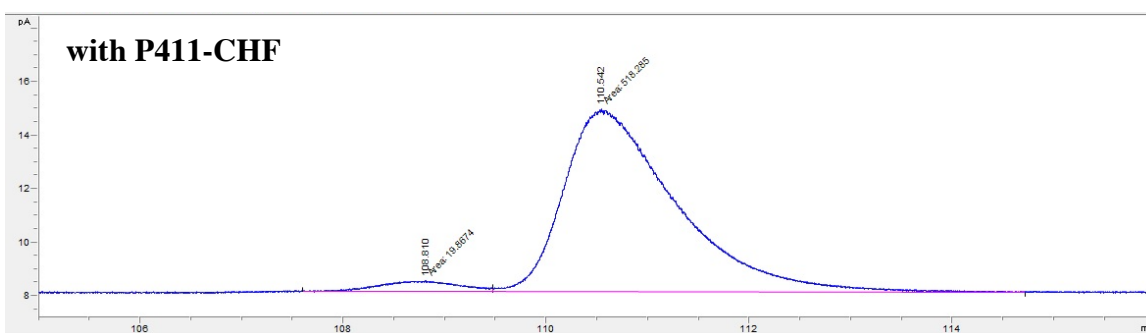
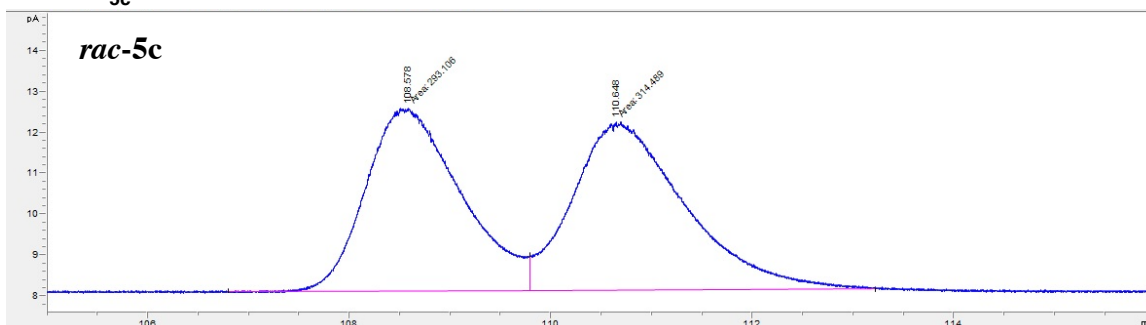


rac-5b			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
45.564	415.3	50.72	45.740	62.0	5.31
48.340	403.5	49.28	48.184	1105.8	94.69
Total	818.8	100.00	Total	1167.8	100.00

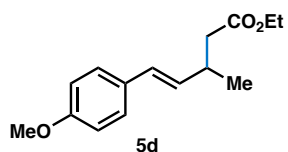


Ethyl (*E*)-9-bromo-3-methoxynon-4-enoate (5c)

GC (CycloSil-B column): 130 °C

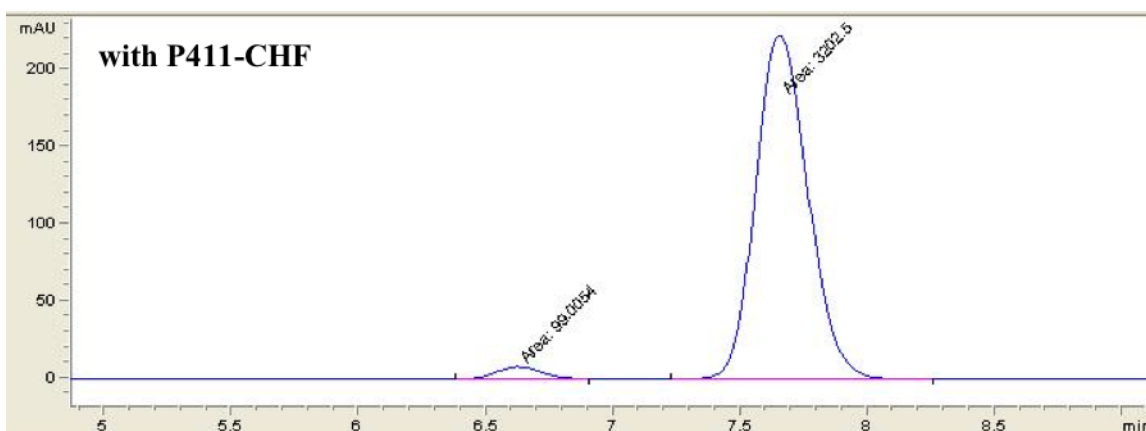
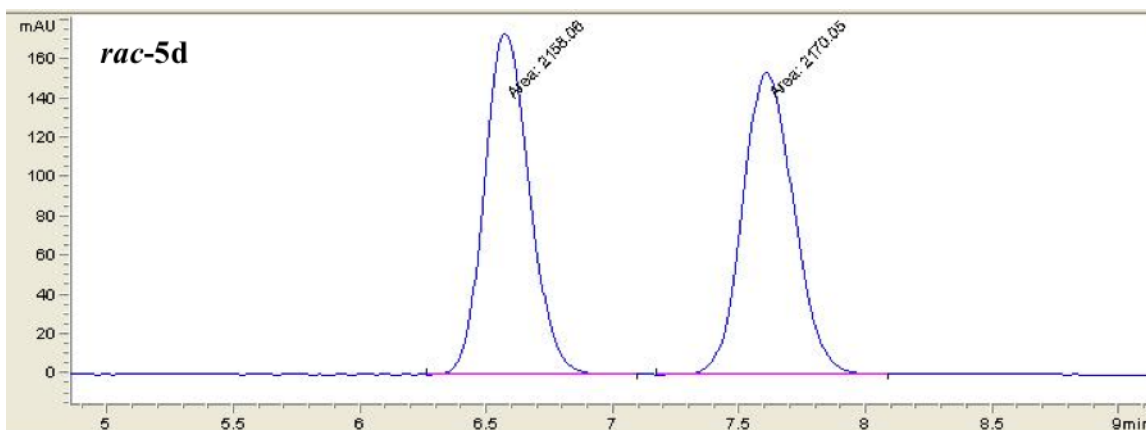


<i>rac</i>-5c			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
108.58	293.1	48.24	108.81	19.90	3.70
110.65	314.5	51.76	110.54	518.30	96.30
Total	607.6	100.00	Total	538.2	100.00

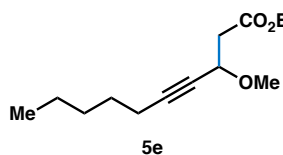


Ethyl (*E*)-5-(4-methoxyphenyl)-3-methylpent-4-enoate (5d)

SFC (Chiralcel OB-H column): 3% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 254 nm.

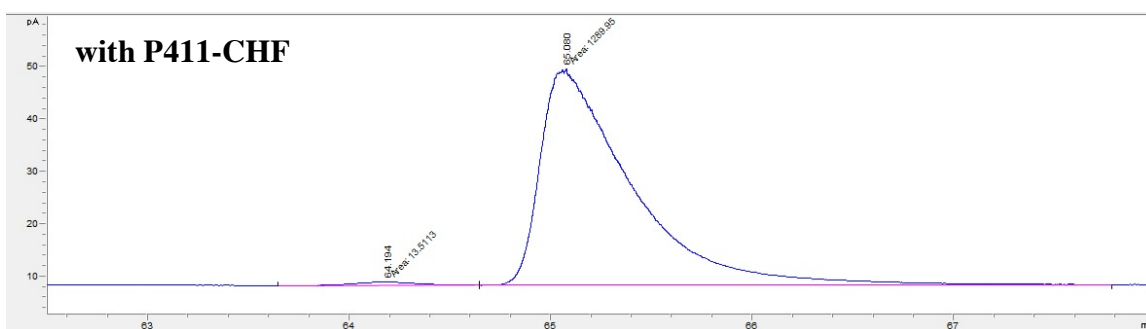
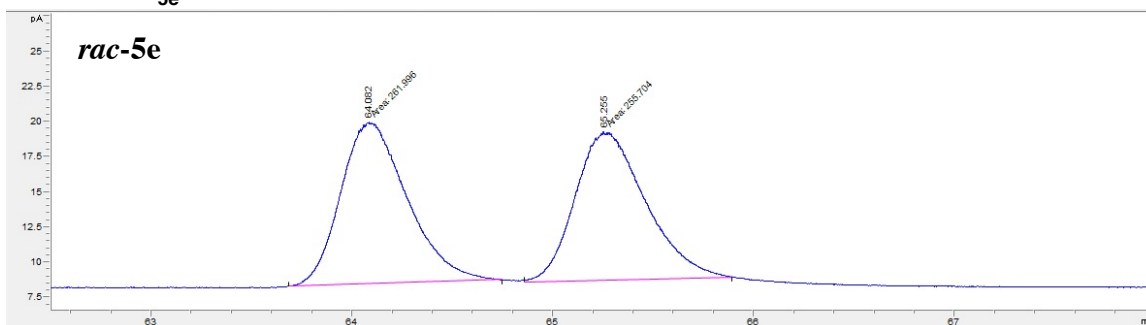


rac-5d			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
6.571	2158.1	49.86	6.624	99.0	3.00
7.605	2170.0	50.14	7.655	3202.5	97.00
Total	4328.1	100.00	Total	3301.5	100.00

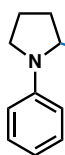


Ethyl 3-methoxydec-4-ynoate (**5e**)

GC (CycloSil-B column): 110 °C for 50 min., ramp 1 °C per minute for 10 min. (to 120 °C), 120 °C for 10 min.

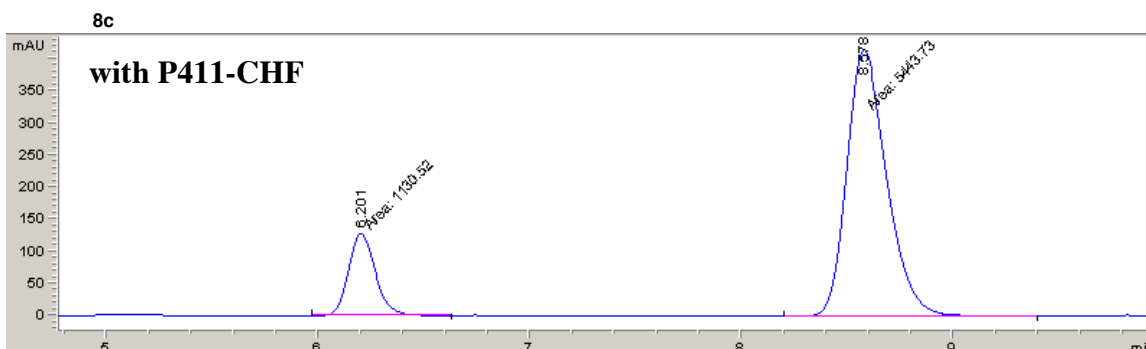


rac-5e			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
64.08	262.0	50.61	64.19	13.5	1.04
65.26	255.7	49.39	65.08	1289.9	98.96
Total	517.7	100.00	Total	1303.4	100.00



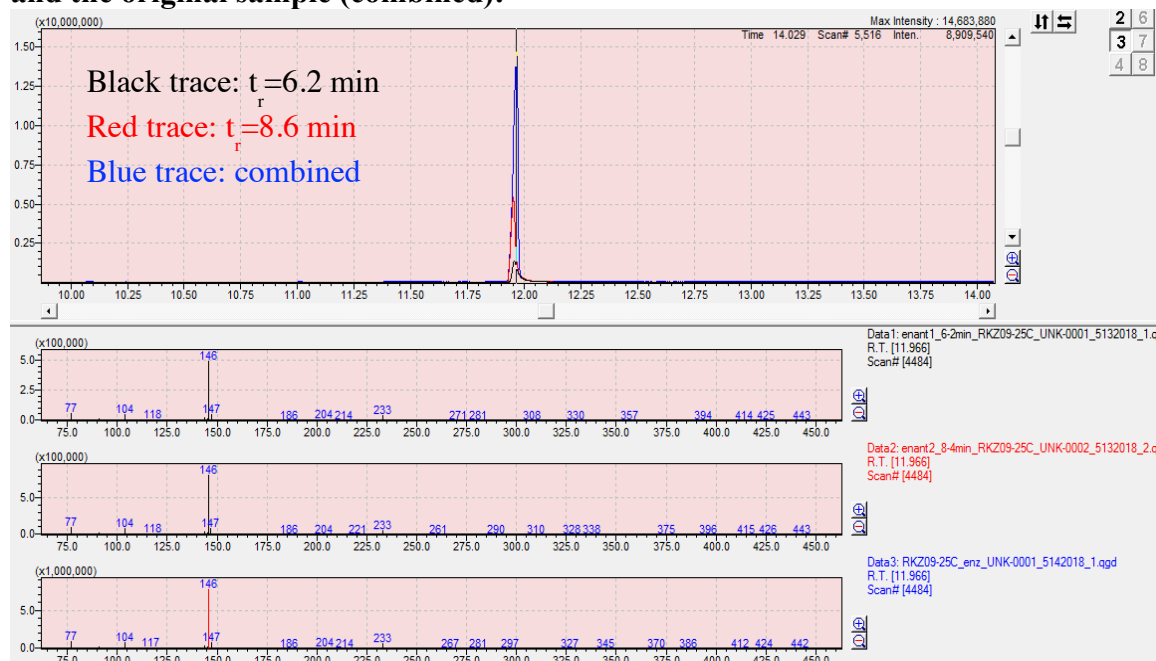
Ethyl 2-(1-phenylpyrrolidin-2-yl)acetate (8c)

HPLC (Chiralcel OD-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, room temperature, 235 nm.

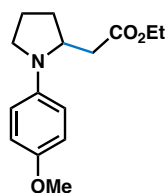


8c with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %
6.201	1130.5	17.20
8.578	5443.7	82.80
Total	6574.2	100.00

GC-MS traces for separated enantiomers ($t_r = 6.2$ min, $t_r = 8.6$ min) individually analyzed and the original sample (combined):

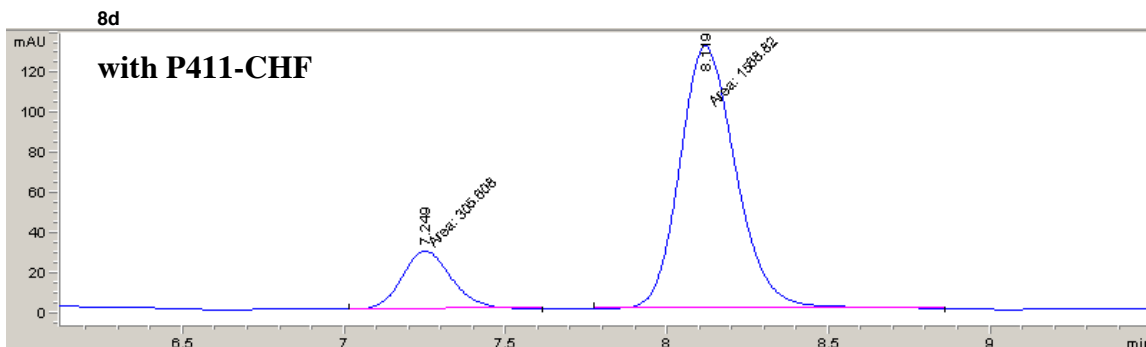


GC-MS conditions (HP-5ms column): 90 °C, isothermal for 2.0 min; 90 °C to 250 °C, 12 °C/min gradient; 250 °C, isothermal for 2.0 min; 250 °C to 300 °C, 30 °C/min gradient; 300 °C, isothermal for 1.0 min.



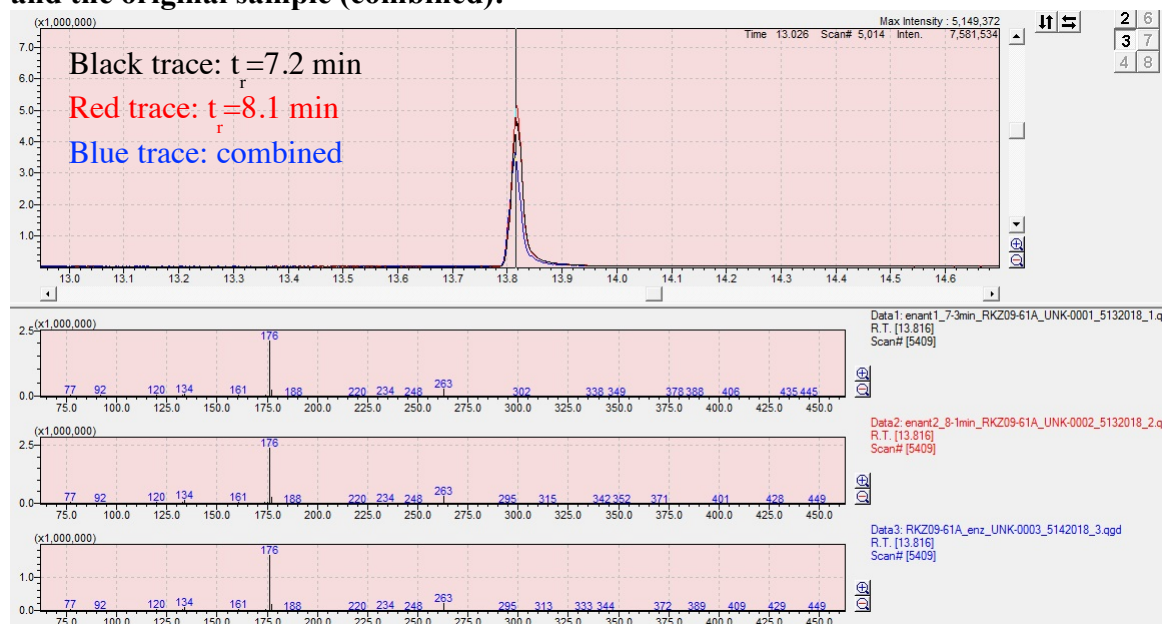
Ethyl 2-(1-(4-methoxyphenyl)pyrrolidin-2-yl)acetate (8d)

HPLC (Chiralcel OD-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, room temperature, 235 nm.

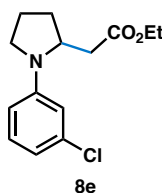


8d with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %
7.249	305.6	16.30
8.119	1568.8	83.70
Total	1874.4	100.00

GC-MS traces for separated enantiomers ($t_r = 7.2$ min, $t_r = 8.1$ min) individually analyzed and the original sample (combined):

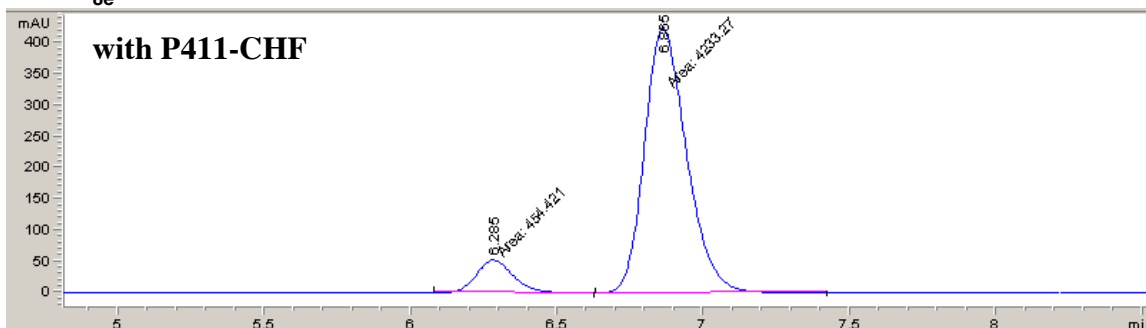


GC-MS conditions (HP-5ms column): 90 °C, isothermal for 2.0 min; 90 °C to 250 °C, 12 °C/min gradient; 250 °C, isothermal for 2.0 min; 250 °C to 300 °C, 30 °C/min gradient; 300 °C, isothermal for 1.0 min.



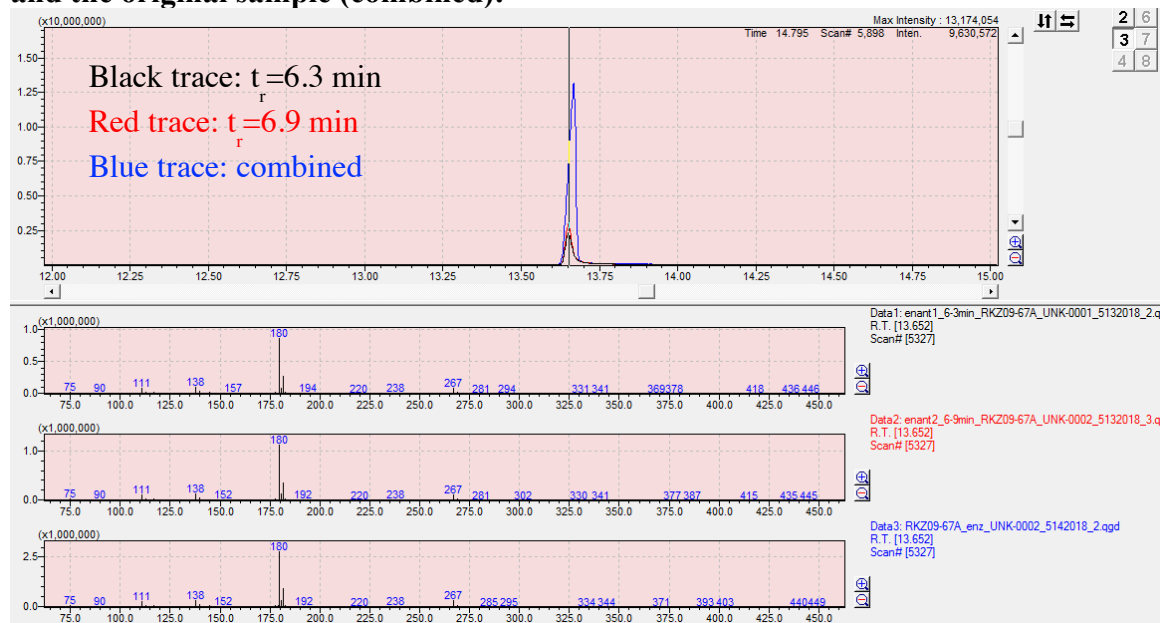
Ethyl 2-(1-(3-chlorophenyl)pyrrolidin-2-yl)acetate (8e)

HPLC (Chiralcel OD-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, room temperature, 235 nm.

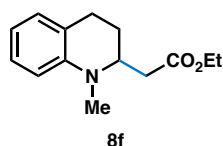


8e with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %
6.285	454.4	9.69
6.865	4233.3	90.31
Total	4687.7	100.00

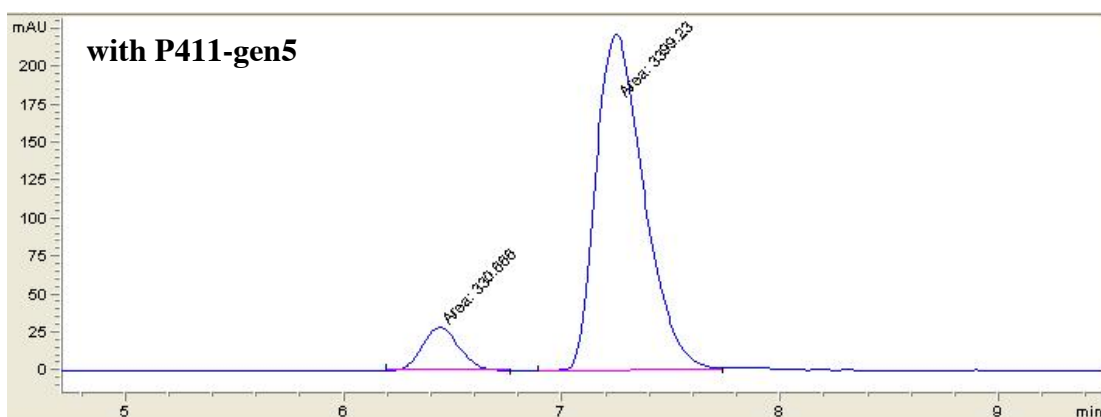
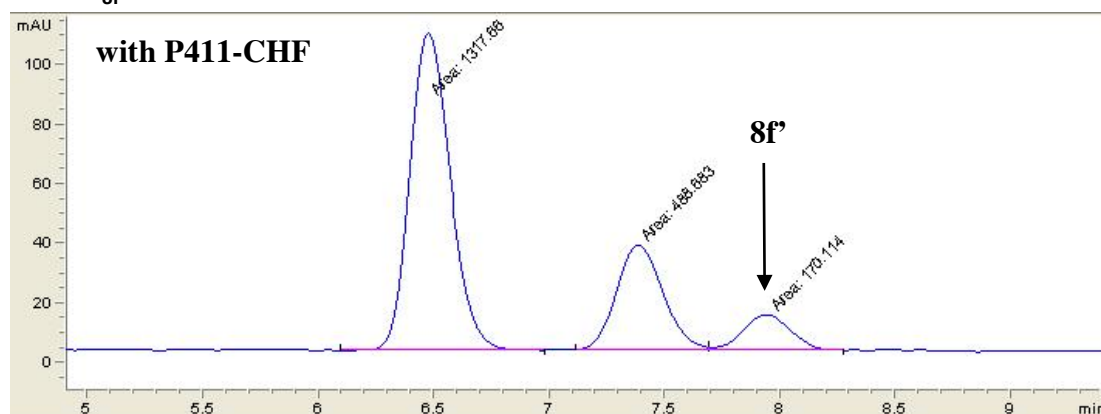
GC-MS traces for separated enantiomers ($t_r = 6.3$ min, $t_r = 6.9$ min) individually analyzed and the original sample (combined):



GC-MS conditions (HP-5ms column): 90 °C, isothermal for 2.0 min; 90 °C to 250 °C, 12 °C/min gradient; 250 °C, isothermal for 2.0 min; 250 °C to 300 °C, 30 °C/min gradient; 300 °C, isothermal for 1.0 min.

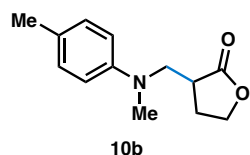


Ethyl 2-(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)acetate (8f)
 SFC (Chiralcel OB-H column): 3% *i*-PrOH in supercritical CO₂, 2.5 mL/min, 40 °C, 235 nm.



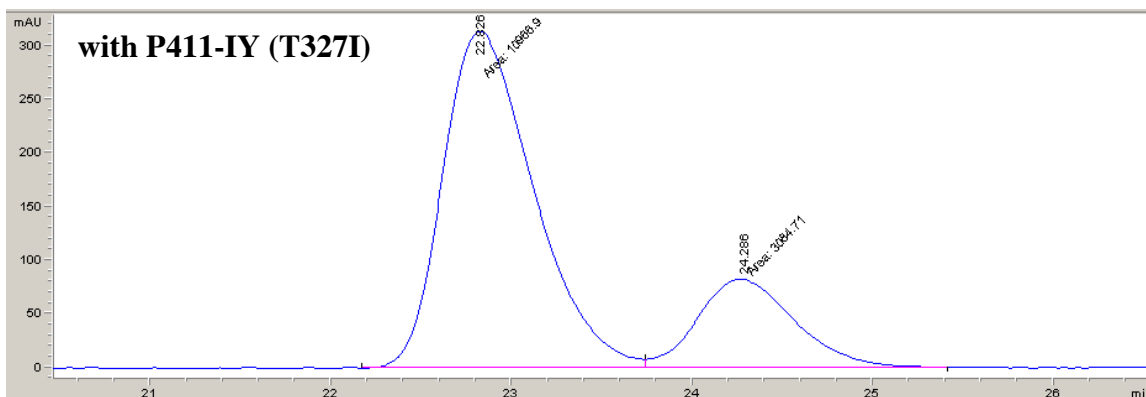
8f with P411-CHF			8f with P411-gen5		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
6.473	1317.7	72.95	6.437	330.7	8.86
7.384	488.7	27.05	7.246	3399.2	91.14
Total	1806.4	100.00	Total	3729.9	100.00

The absolute configuration of P411-gen5 synthesized (+)-**8f** was determined to be (*S*) after derivatization to (+)-cuspateine (see **Section X** for more details). As P411-CHF shows the opposite selectivity for the synthesis of (–)-**8f**, P411-CHF produces (*R*)-**8f** in excess.



3-((Methyl(*p*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (10b)

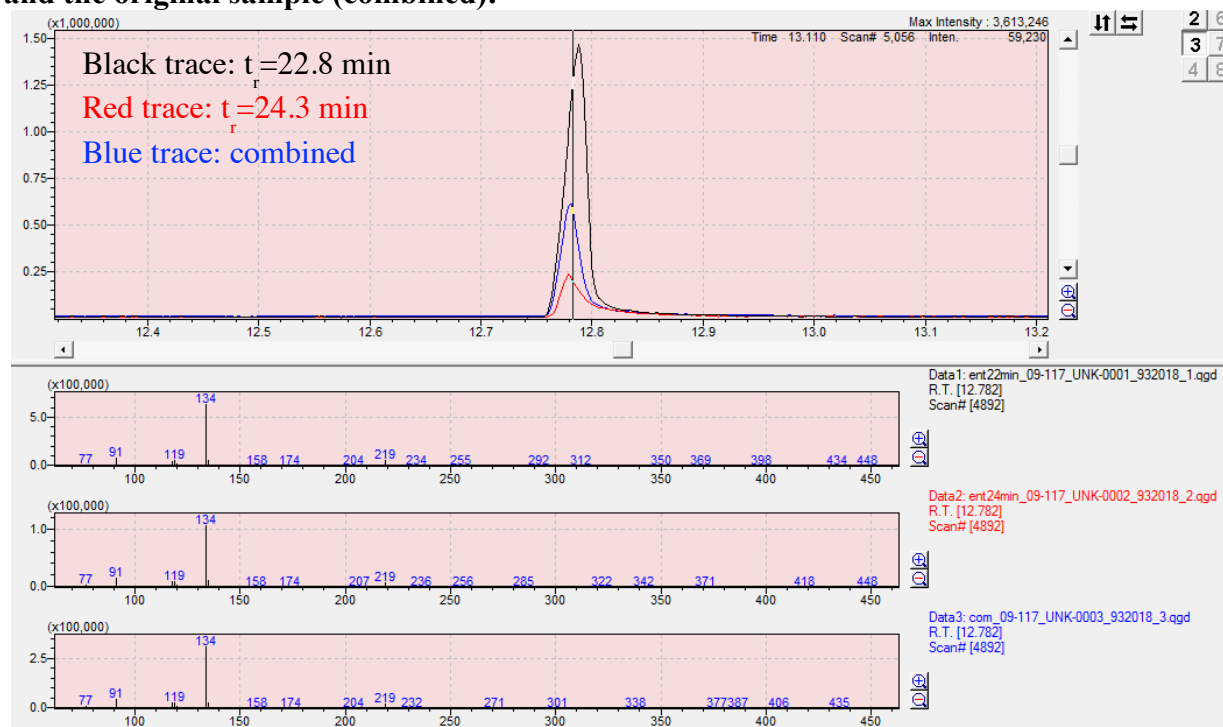
HPLC (Chiralcel OD-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, 32 °C, 254 nm.



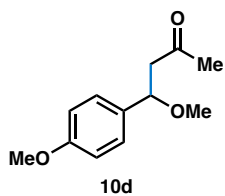
10b with P411-IY (T327I)

Retention Time (min)	Area (mAU*s)	Area %
22.826	10966.9	78.05
24.286	3084.7	21.95
Total	14051.6	100.00

GC-MS traces for separated enantiomers ($t_r = 22.8$ min, $t_r = 24.3$ min) individually analyzed and the original sample (combined):

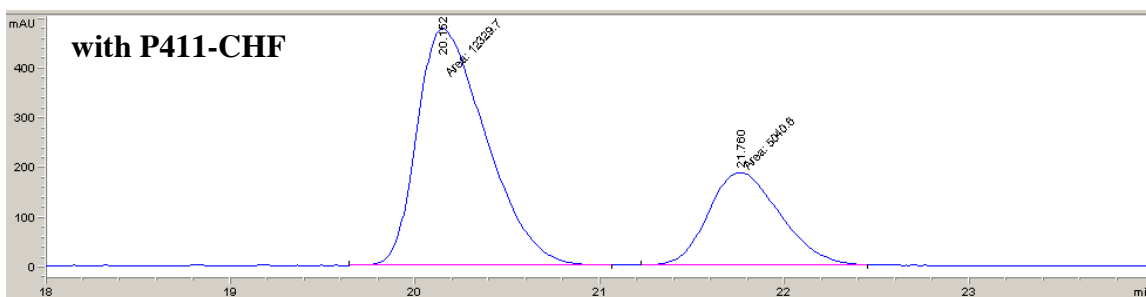
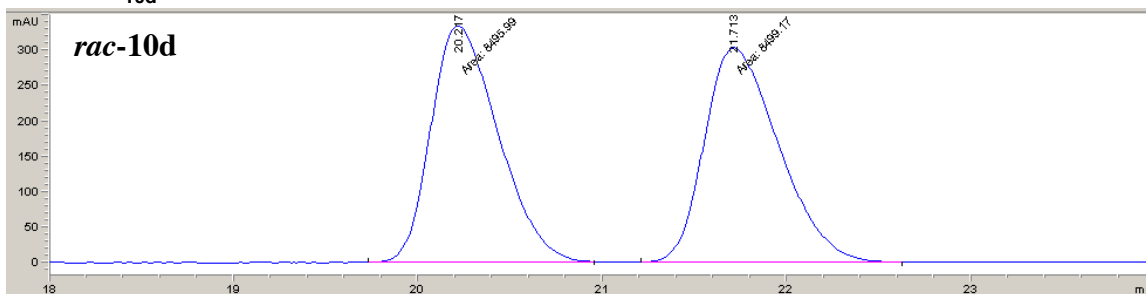


GC-MS conditions (HP-5ms column): 90 °C, isothermal for 2.0 min; 90 °C to 250 °C, 12 °C/min gradient; 250 °C, isothermal for 2.0 min; 250 °C to 300 °C, 30 °C/min gradient; 300 °C, isothermal for 1.0 min.



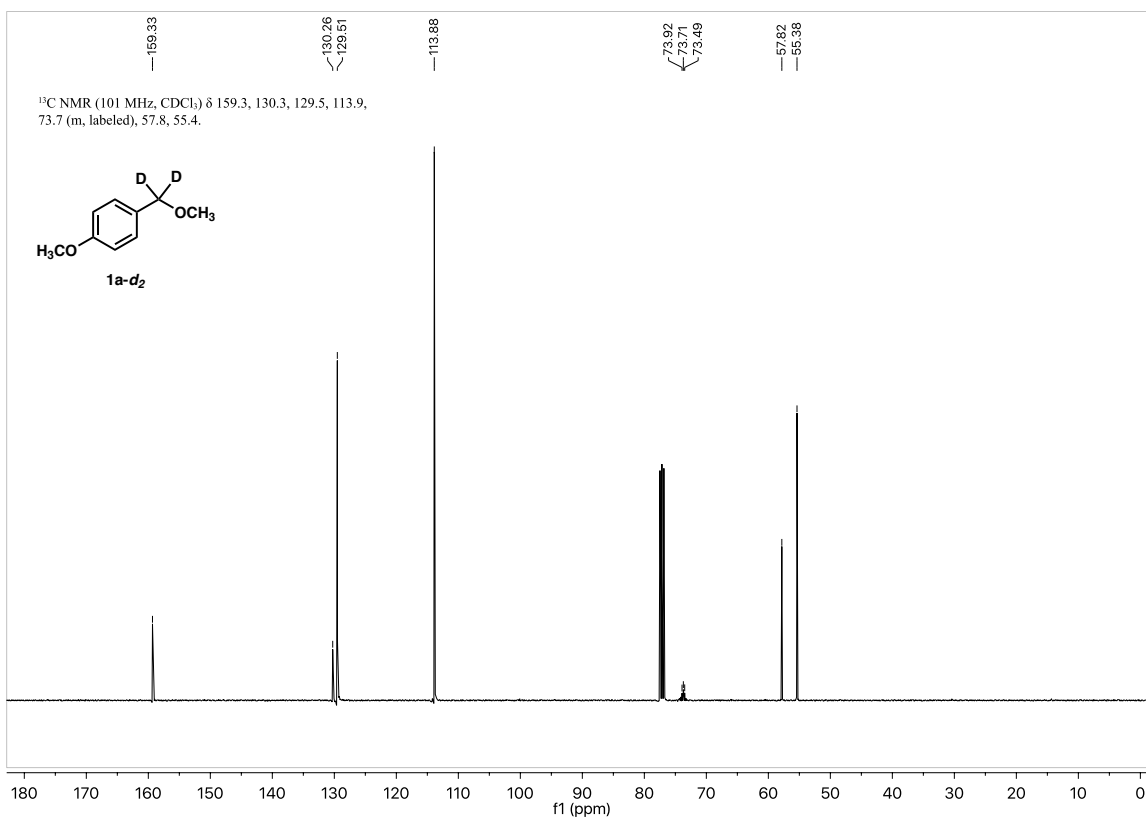
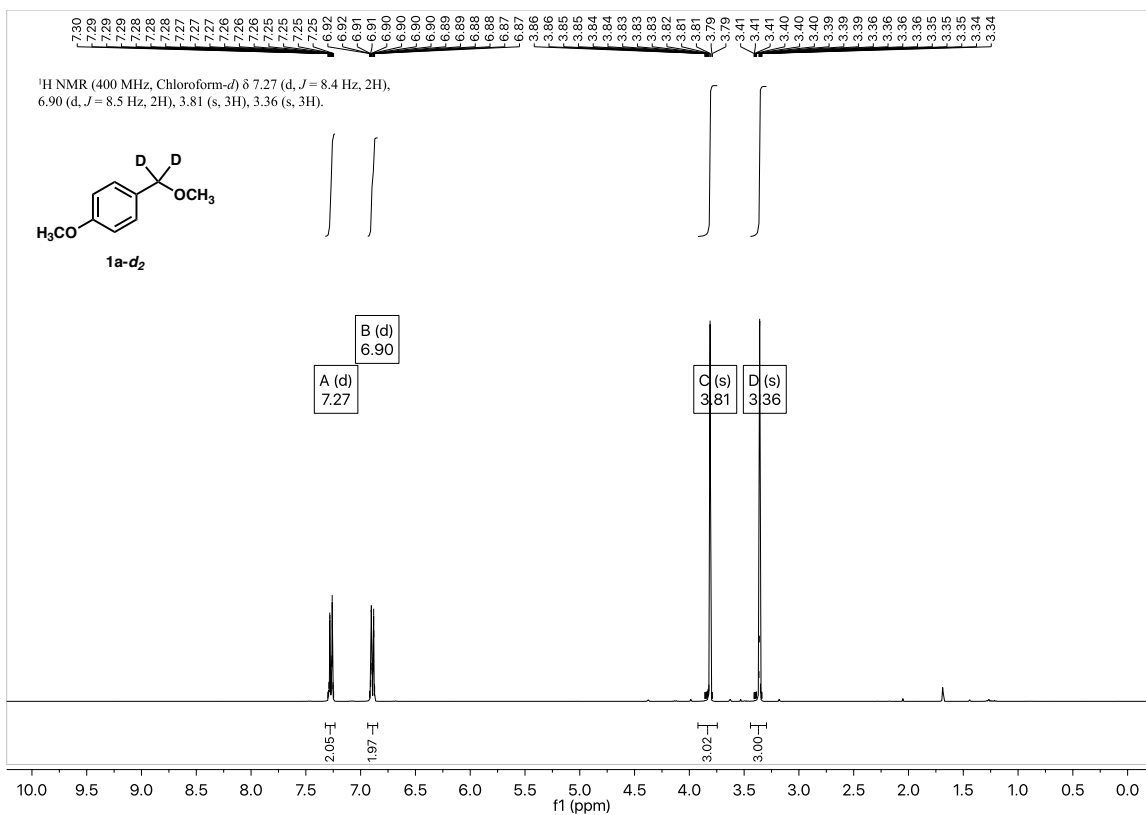
4-Methoxy-4-(4-methoxyphenyl)butan-2-one (10d)

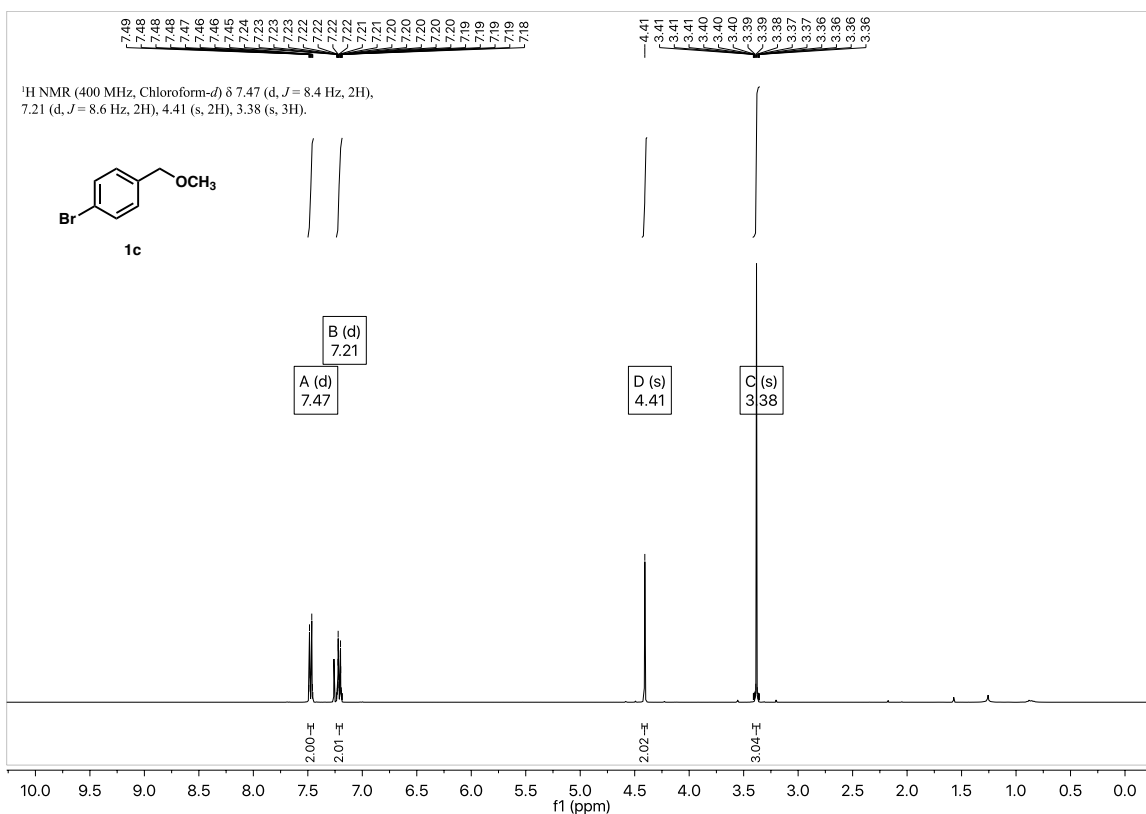
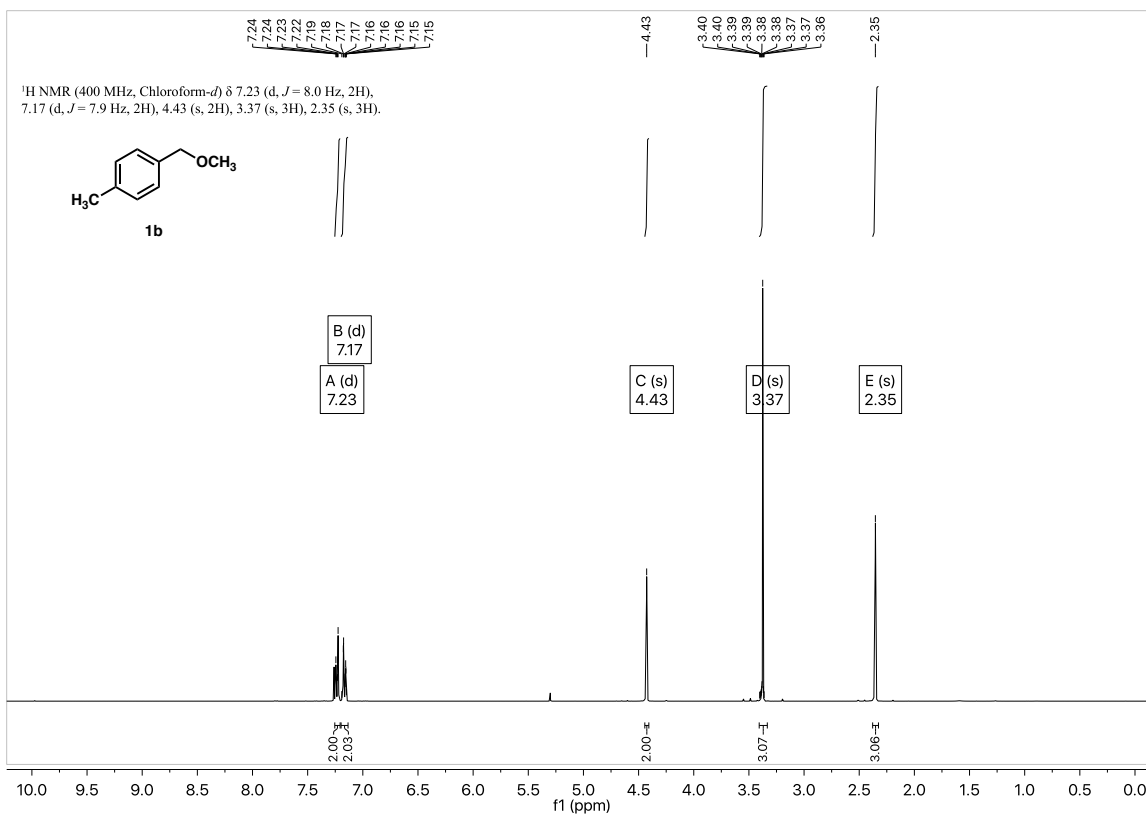
HPLC (Chiralcel OJ-H column): 6% *i*-PrOH in hexane, 1.0 mL/min, 28 °C, 220 nm.

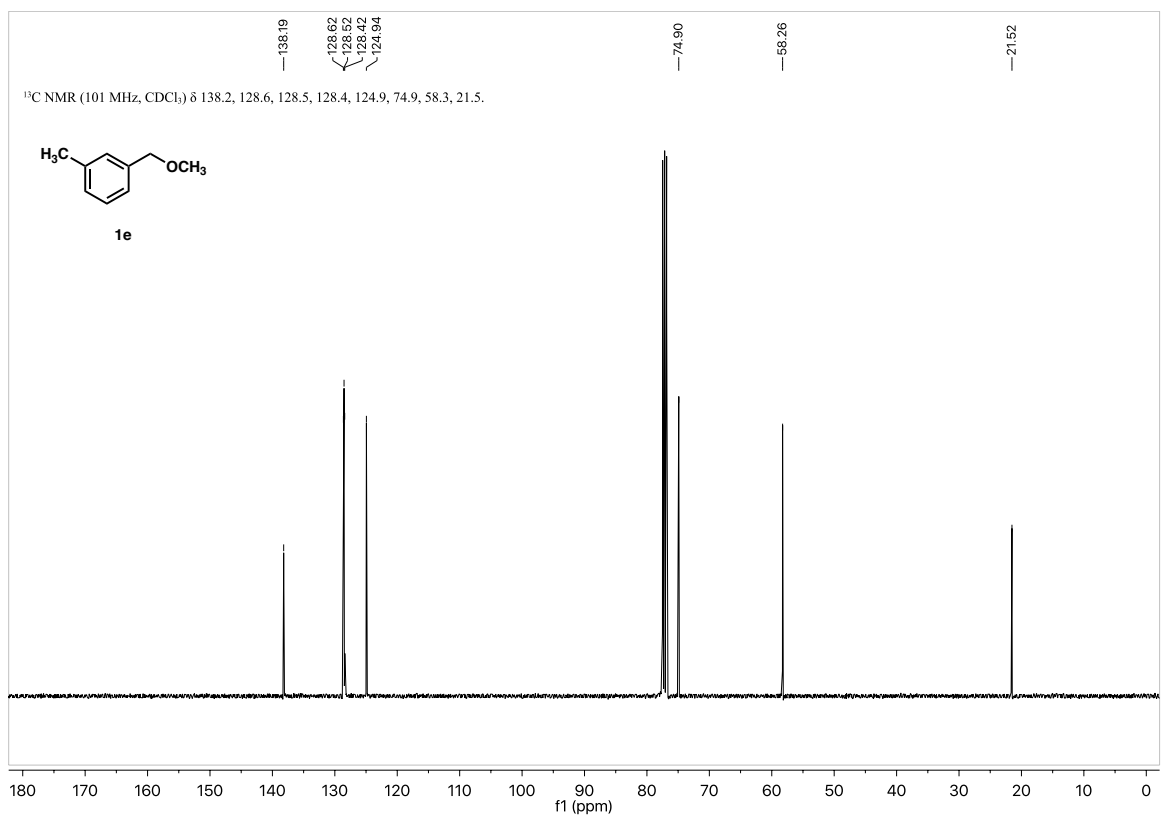
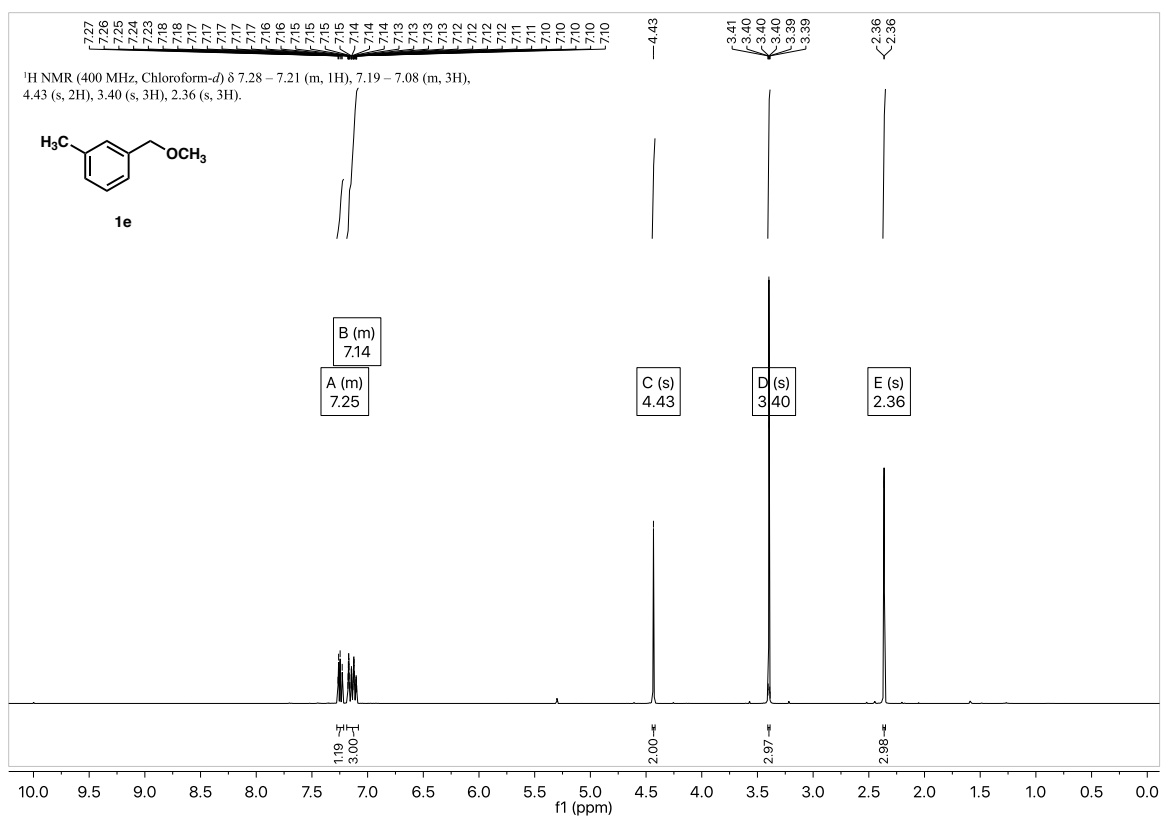


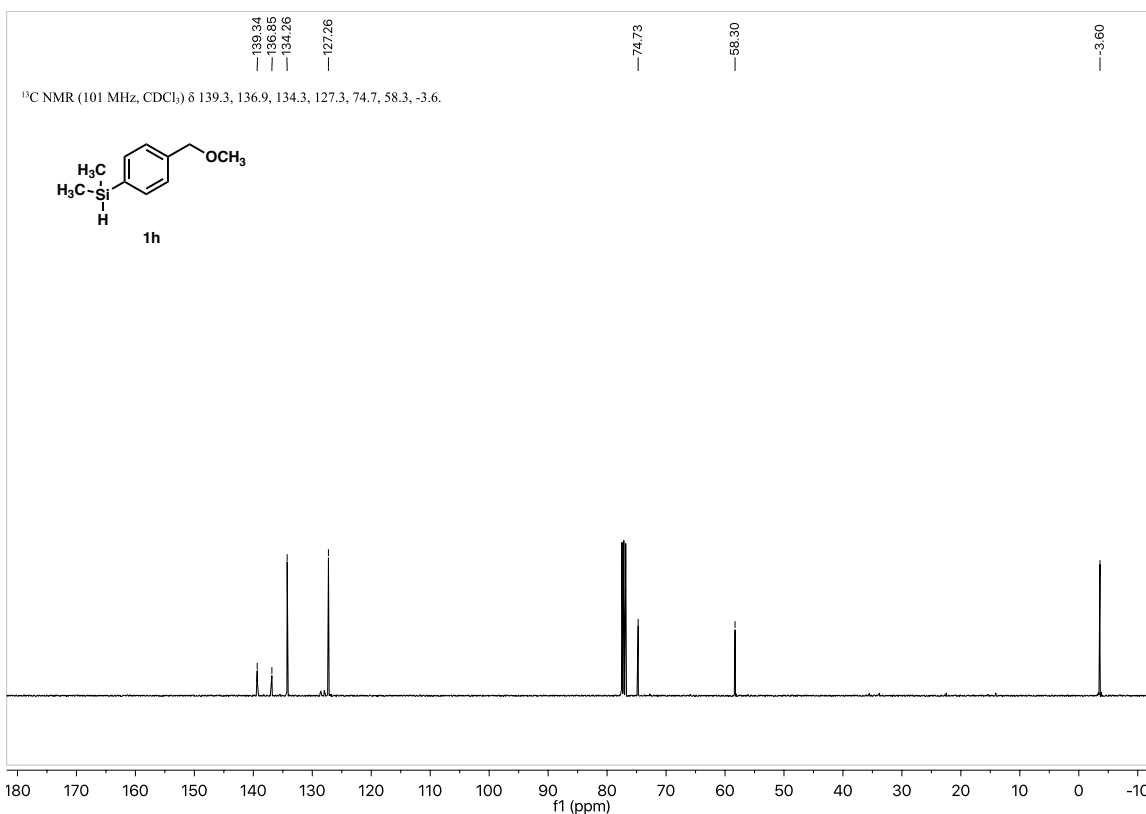
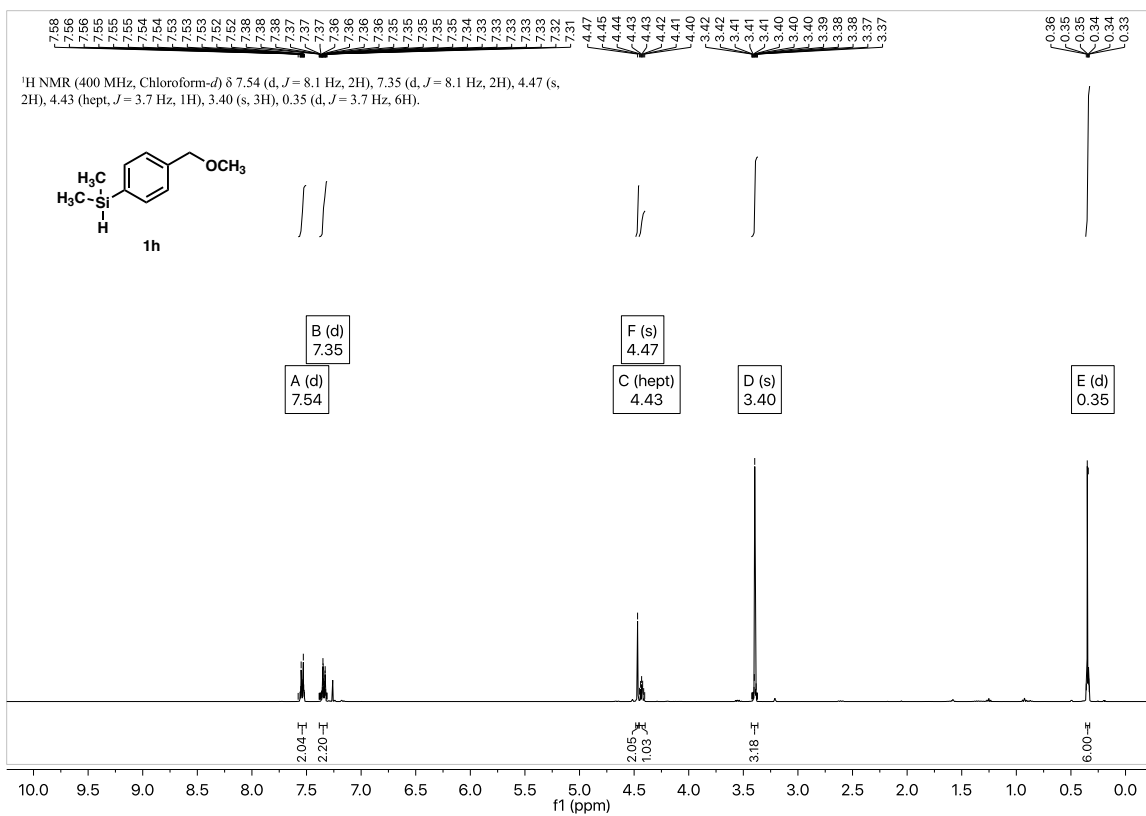
rac-10d			with P411-CHF		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
20.217	8495.99	49.99	20.152	12329.7	70.98
21.713	8499.17	50.01	21.760	5040.6	29.02
Total	16995.16	100.00	Total	17370.3	100.00

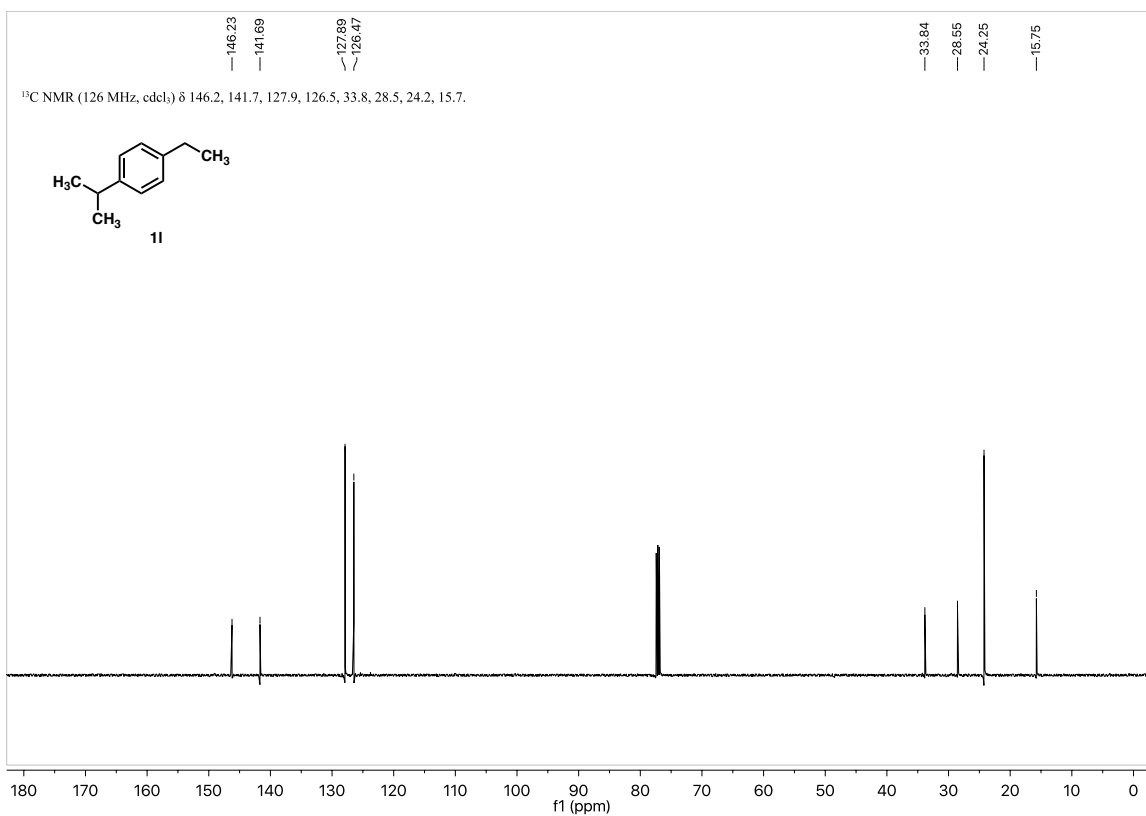
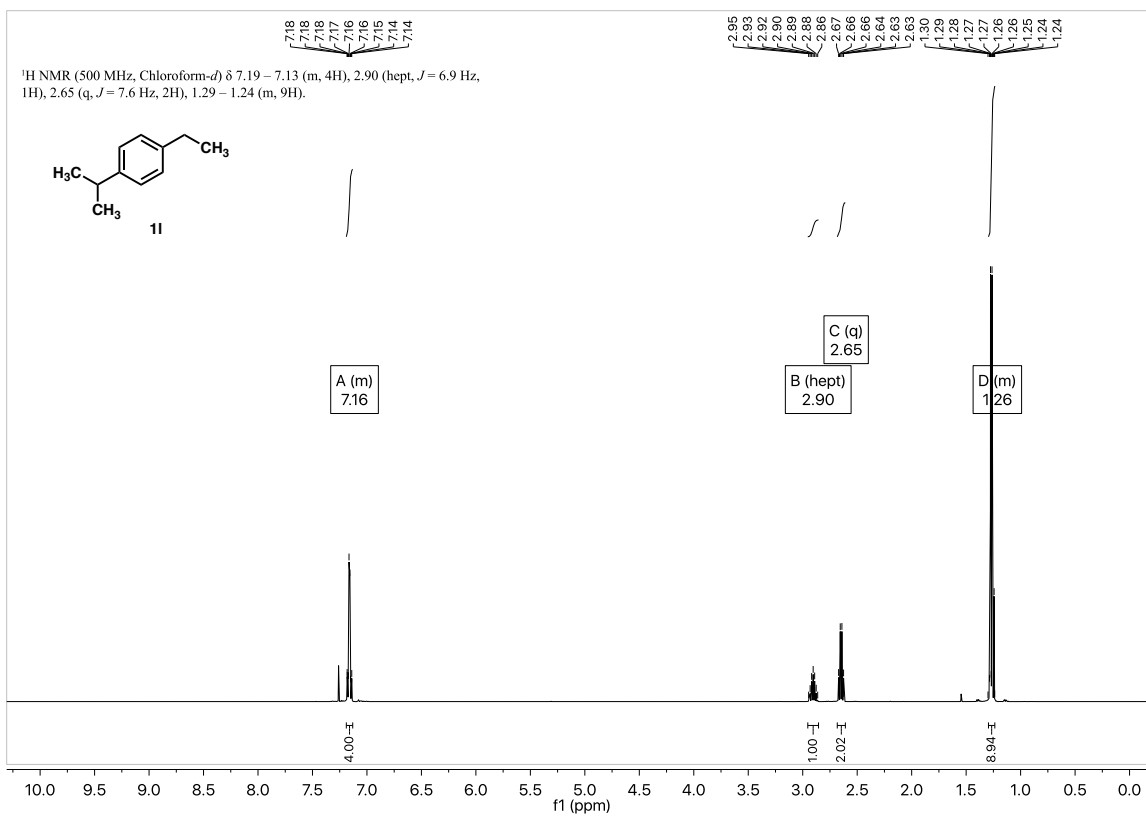
XII. ^1H and ^{13}C NMR Spectra

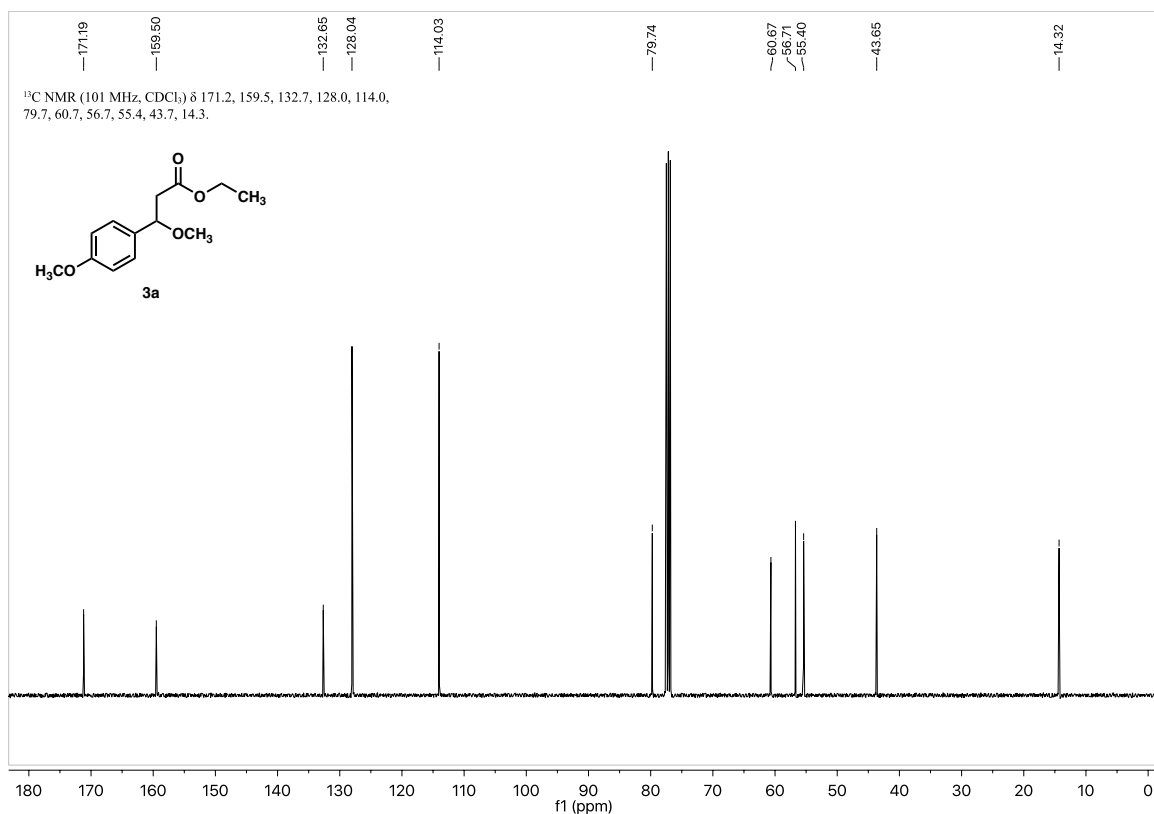
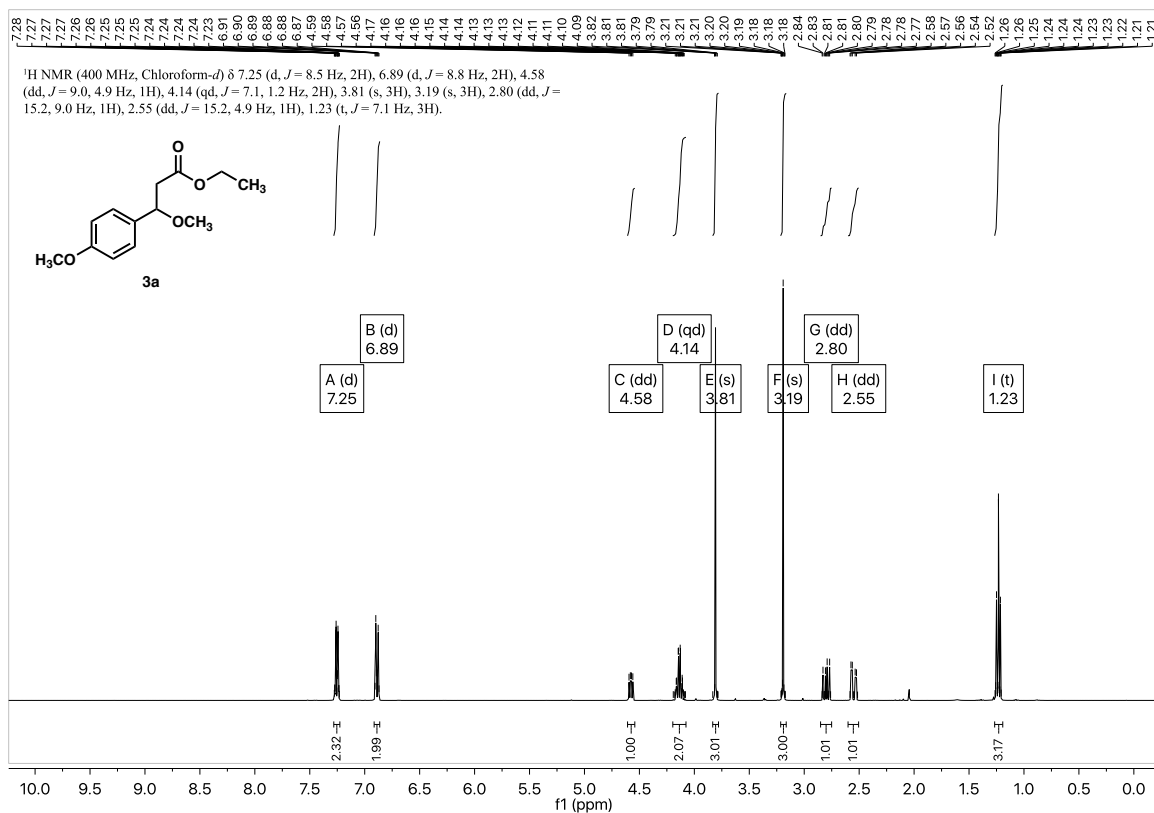


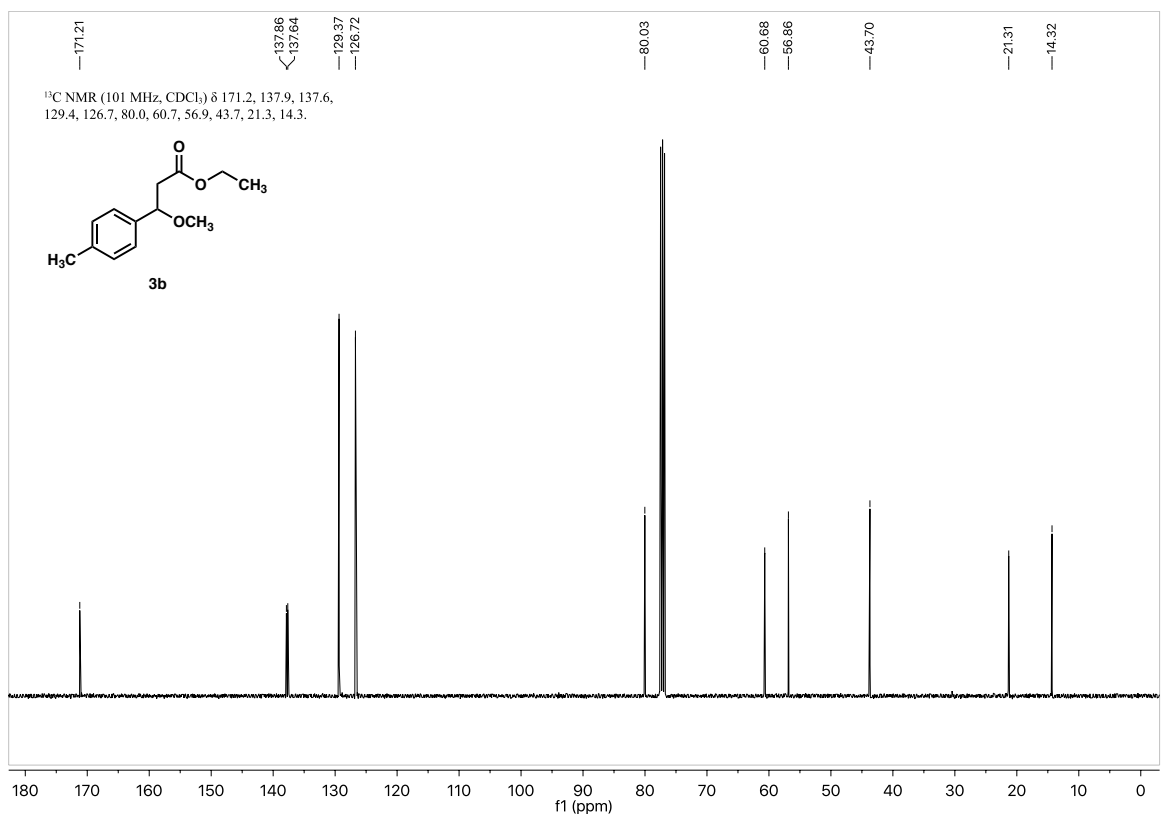
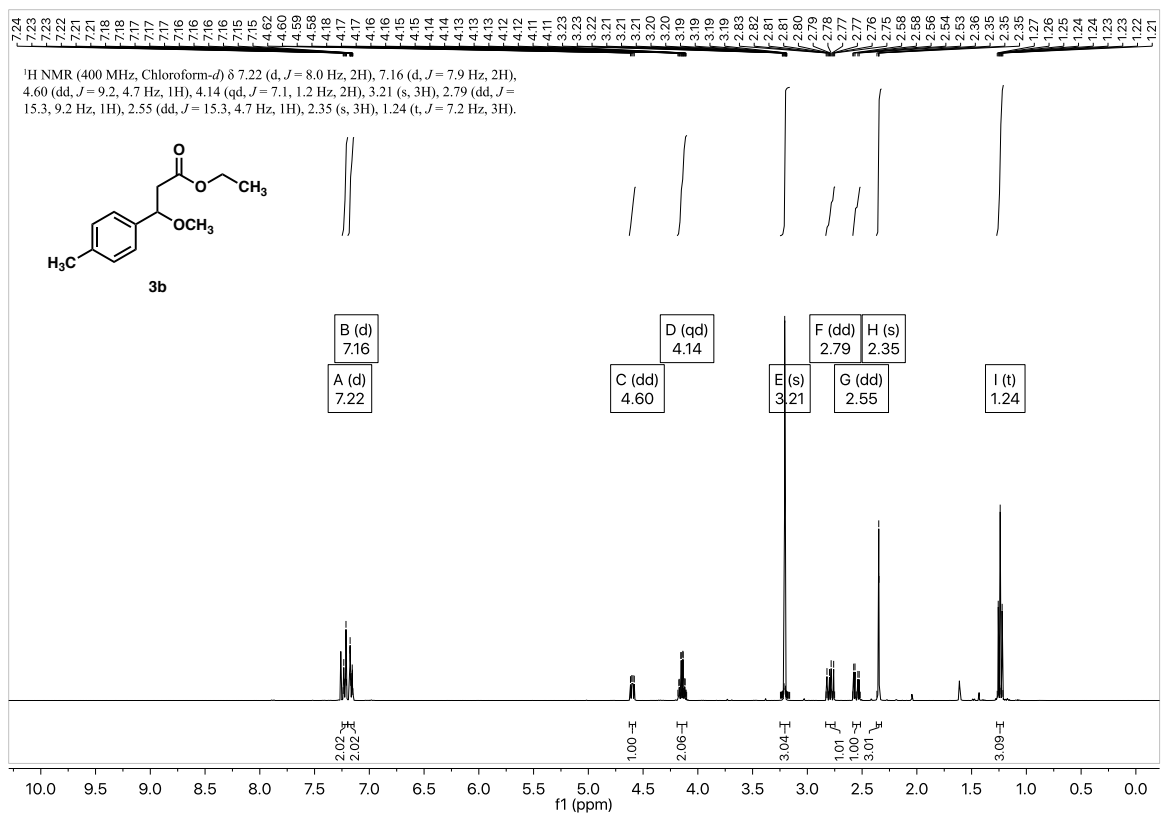


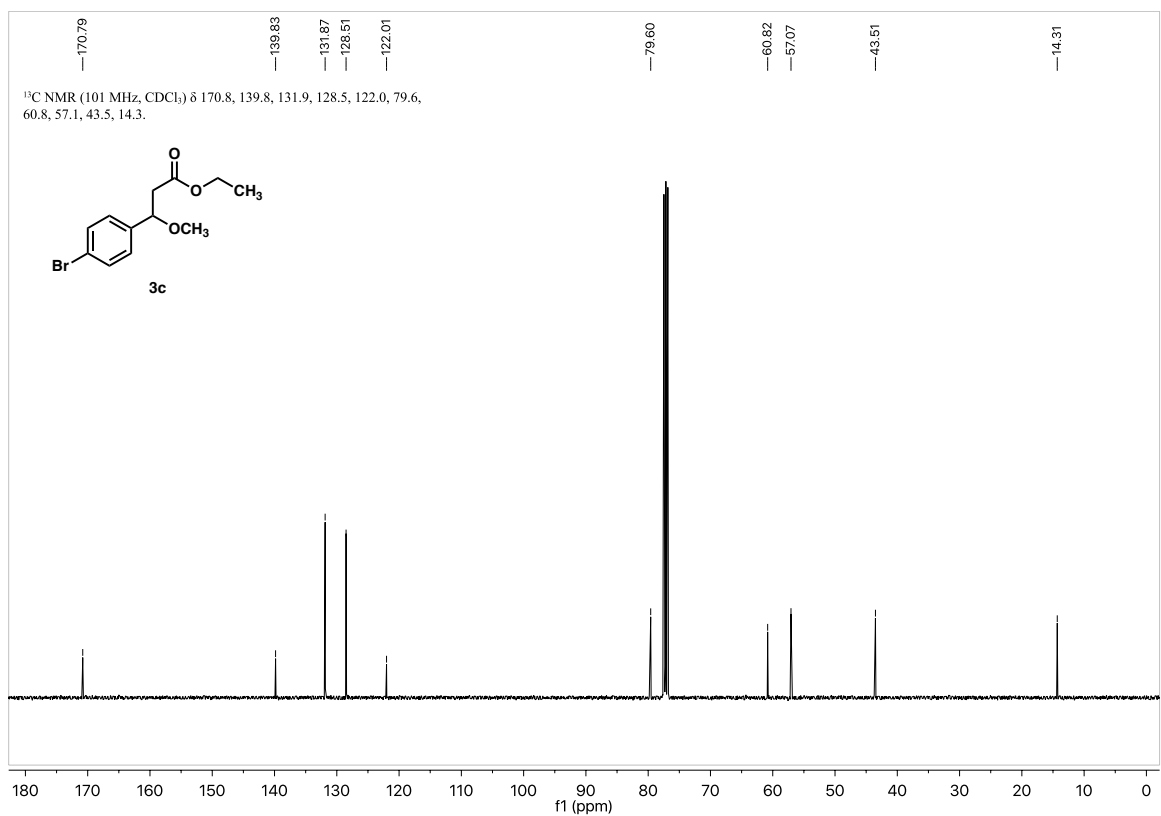
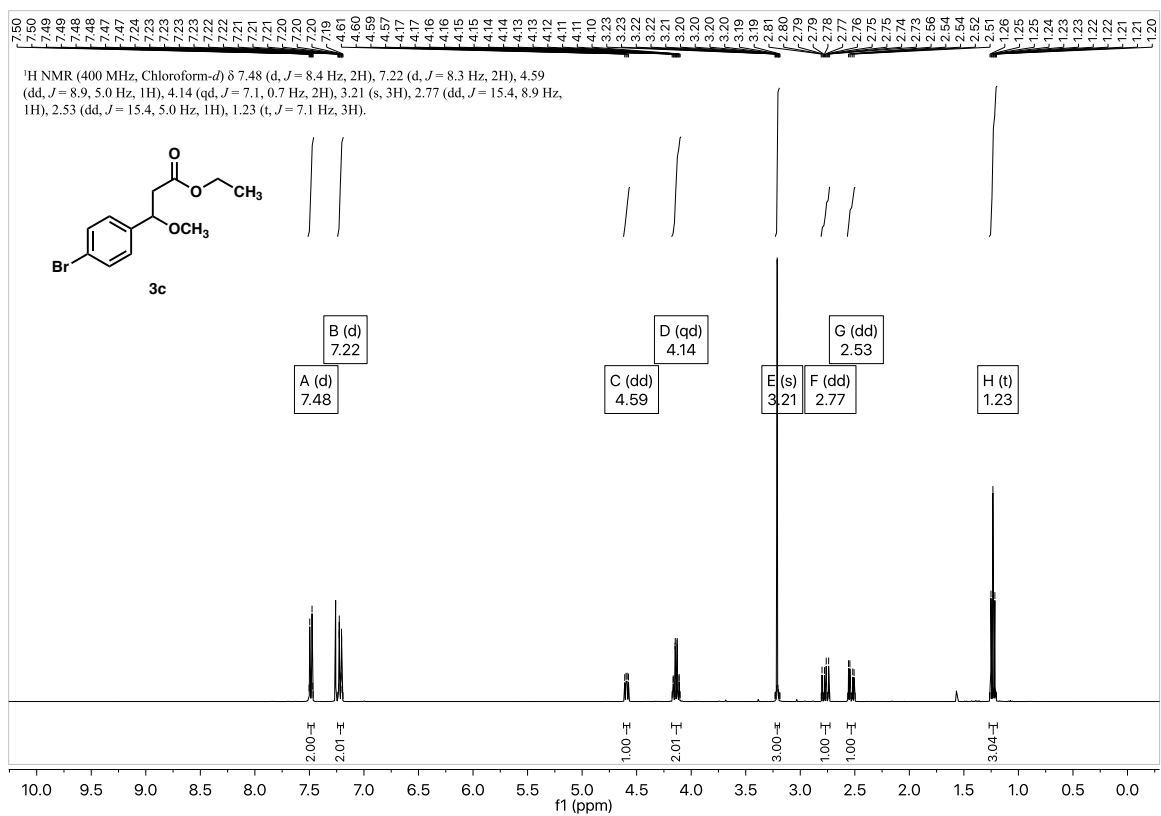


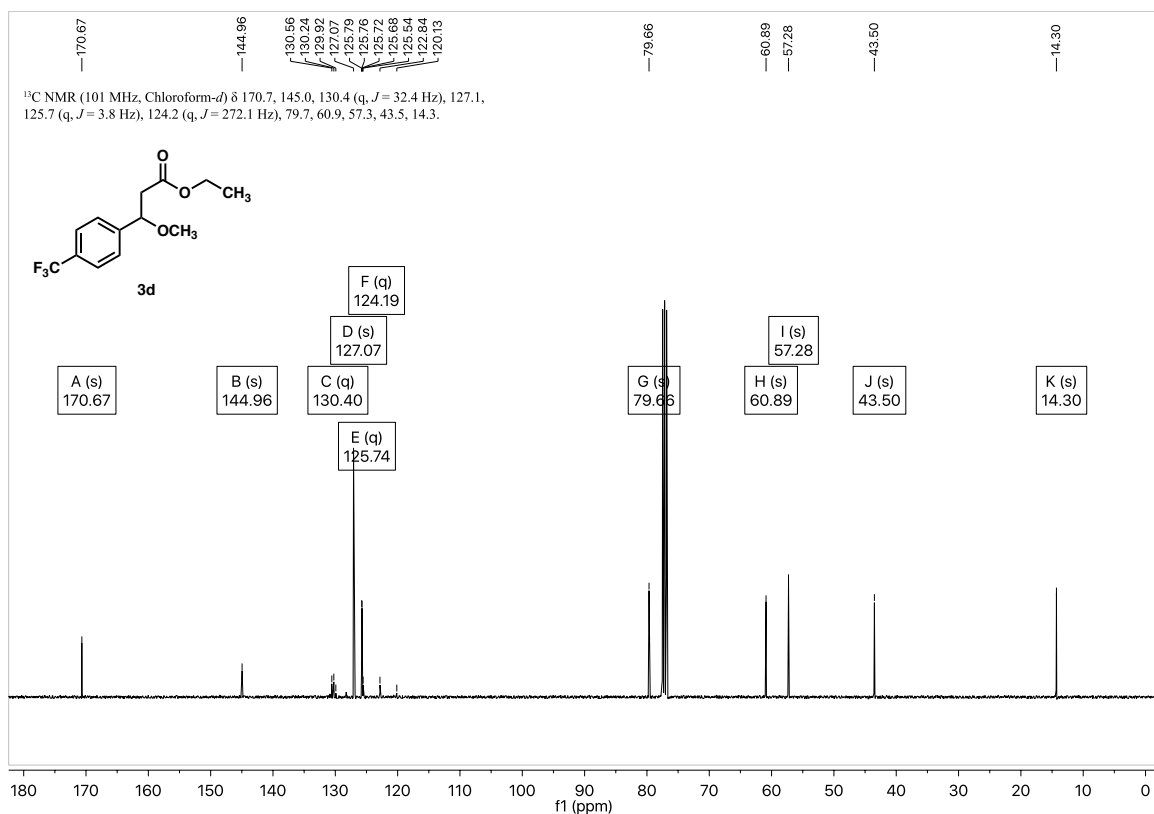
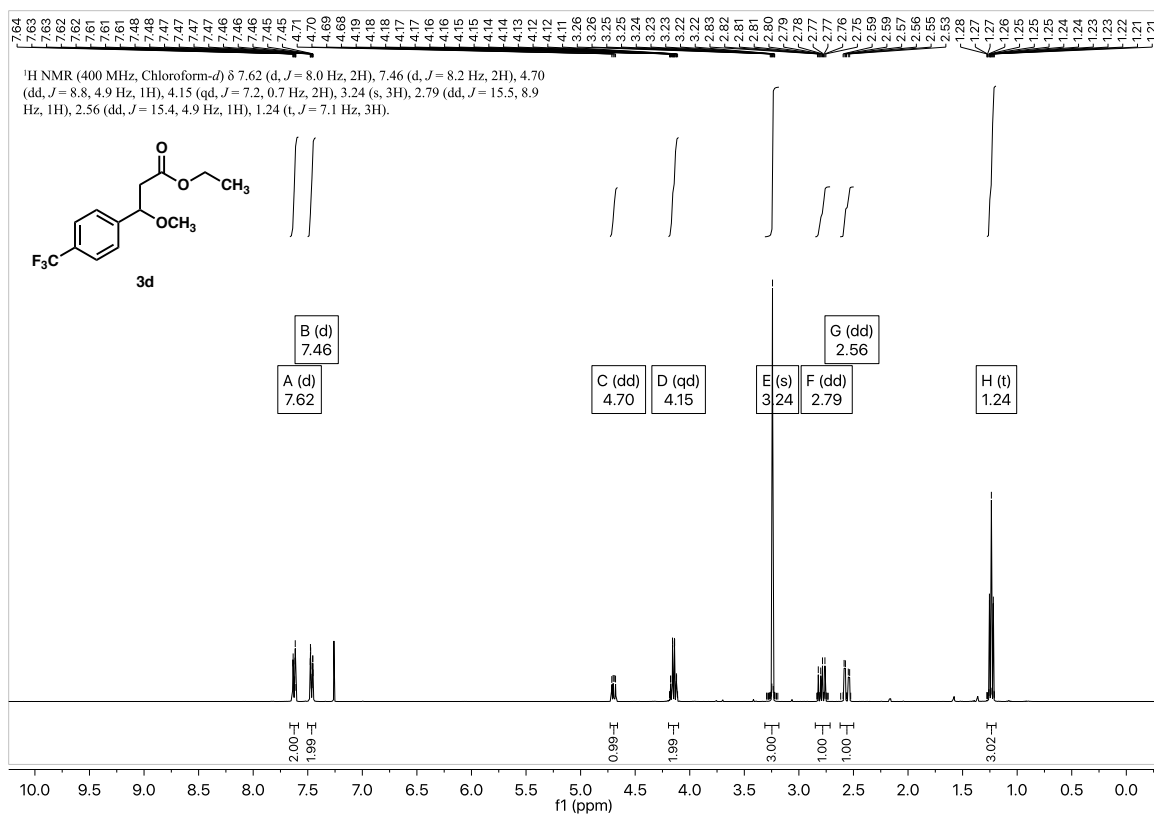


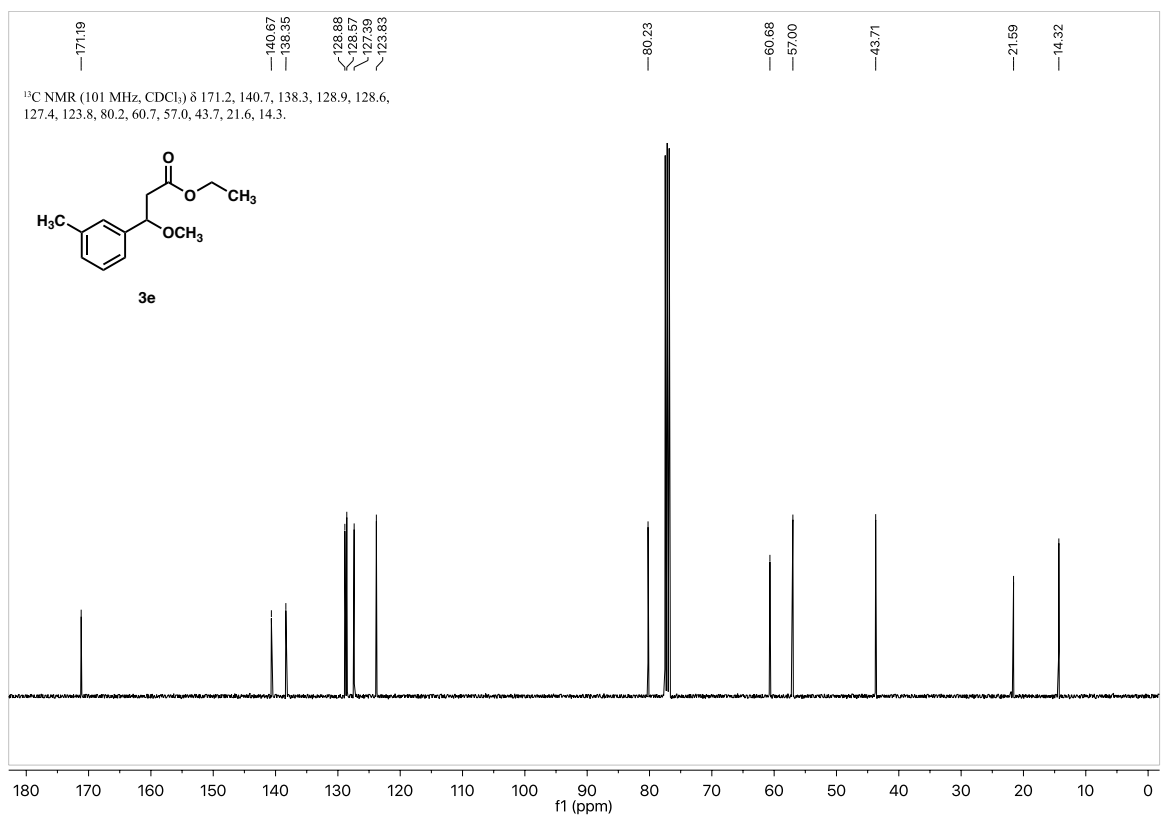
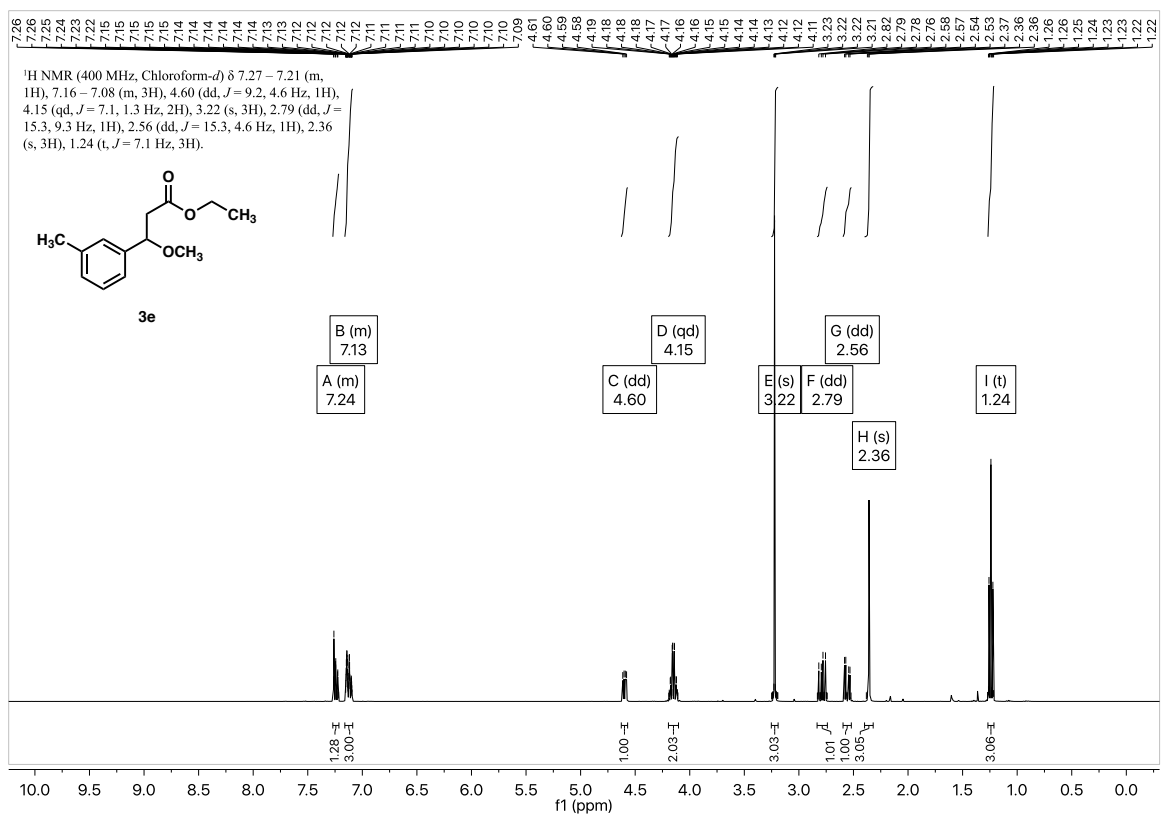


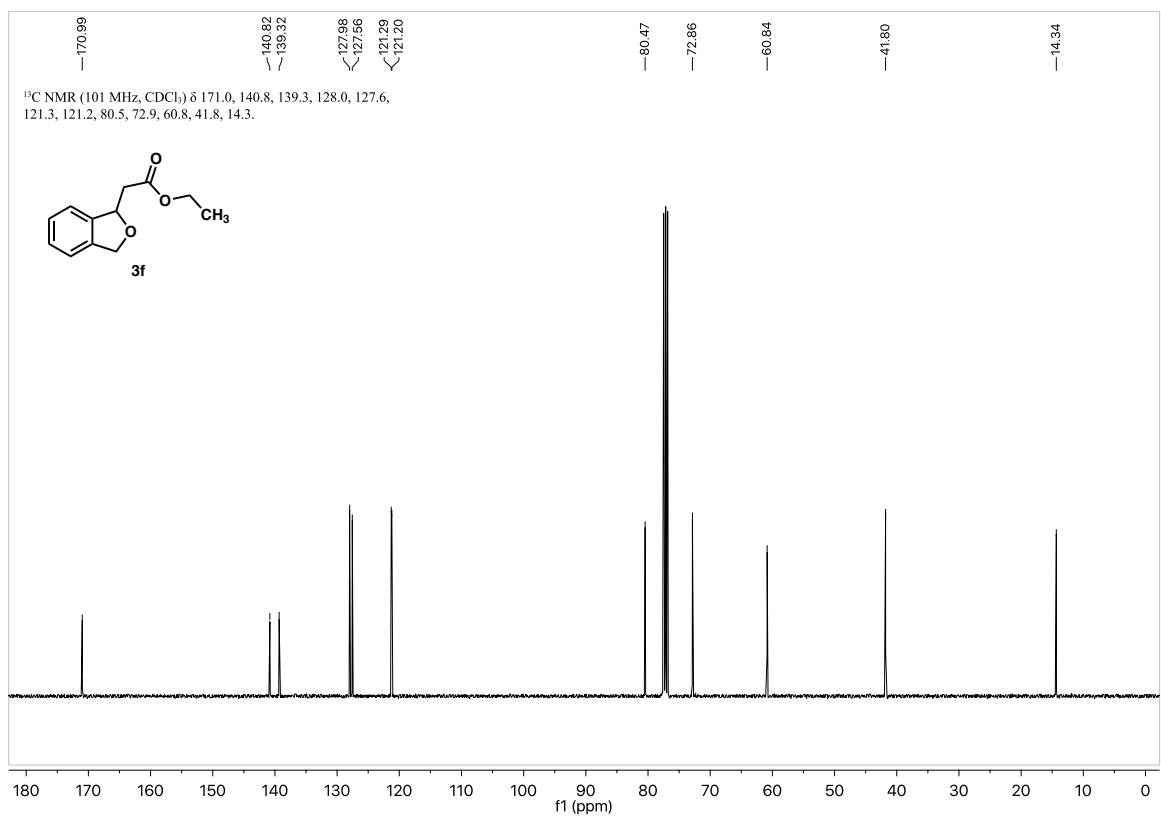
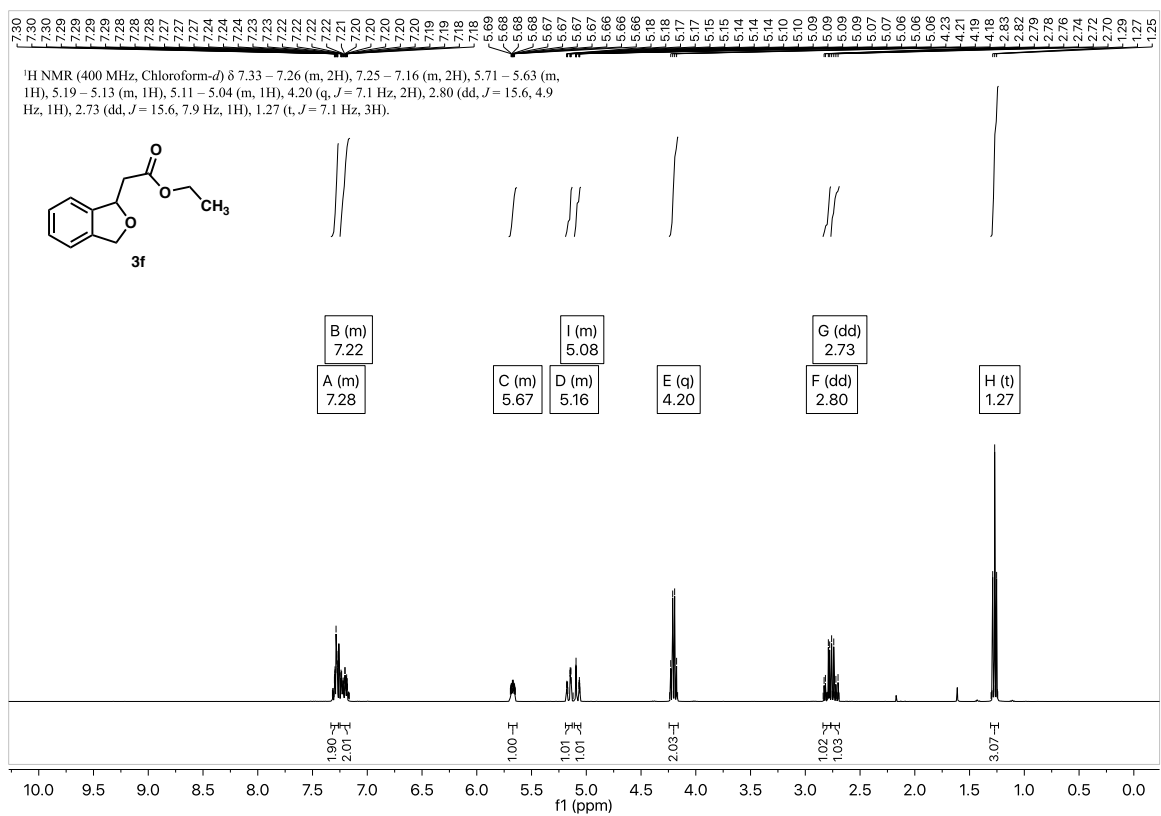


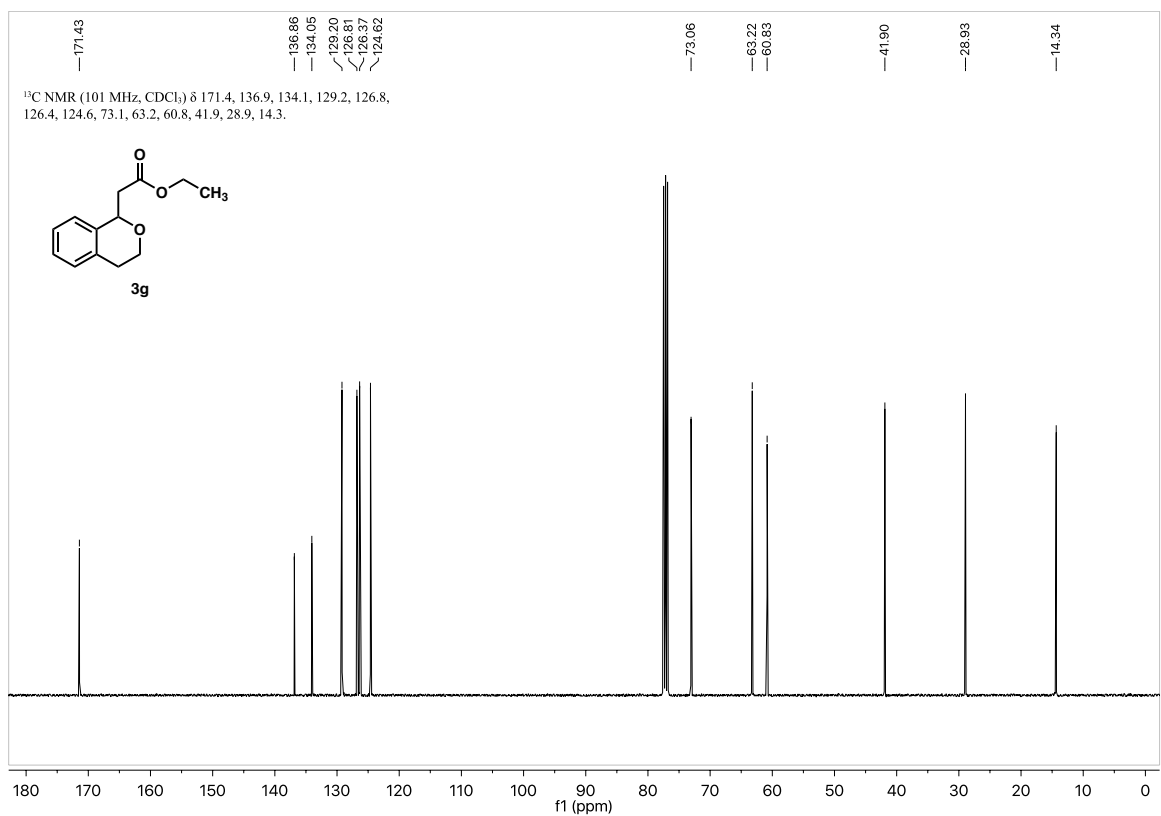
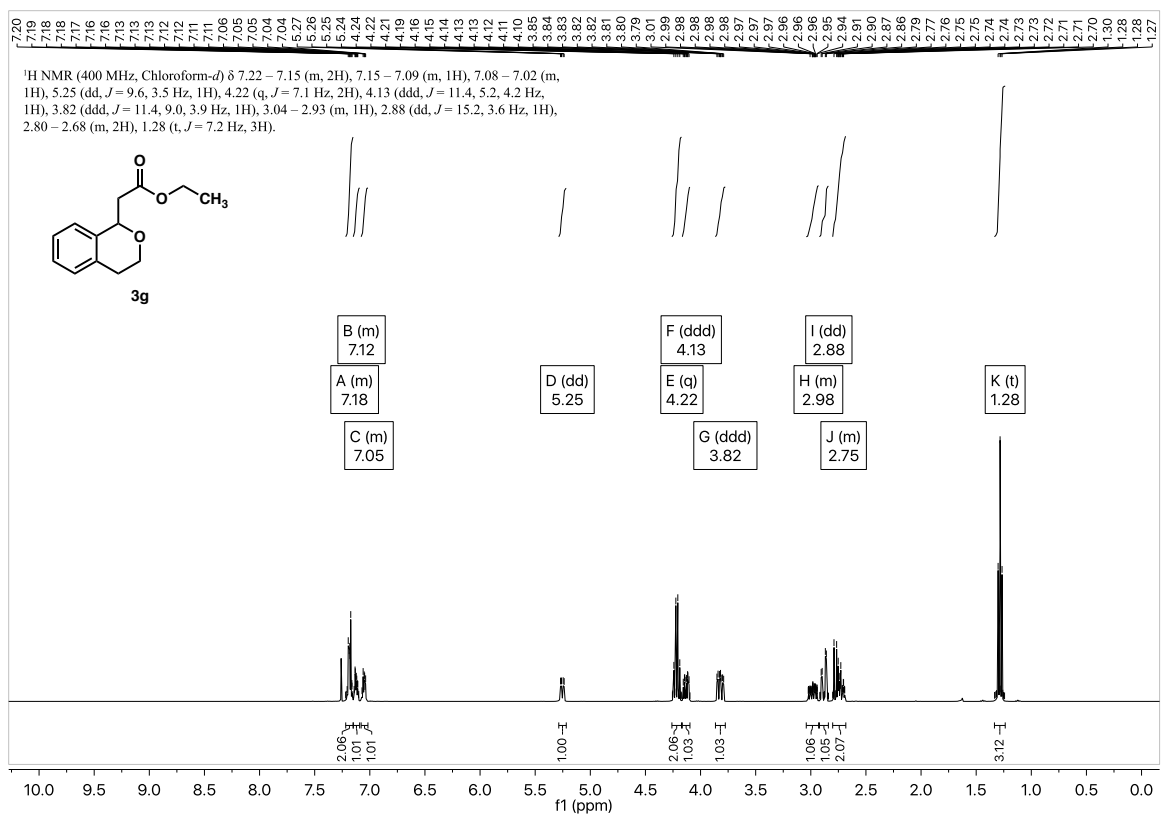


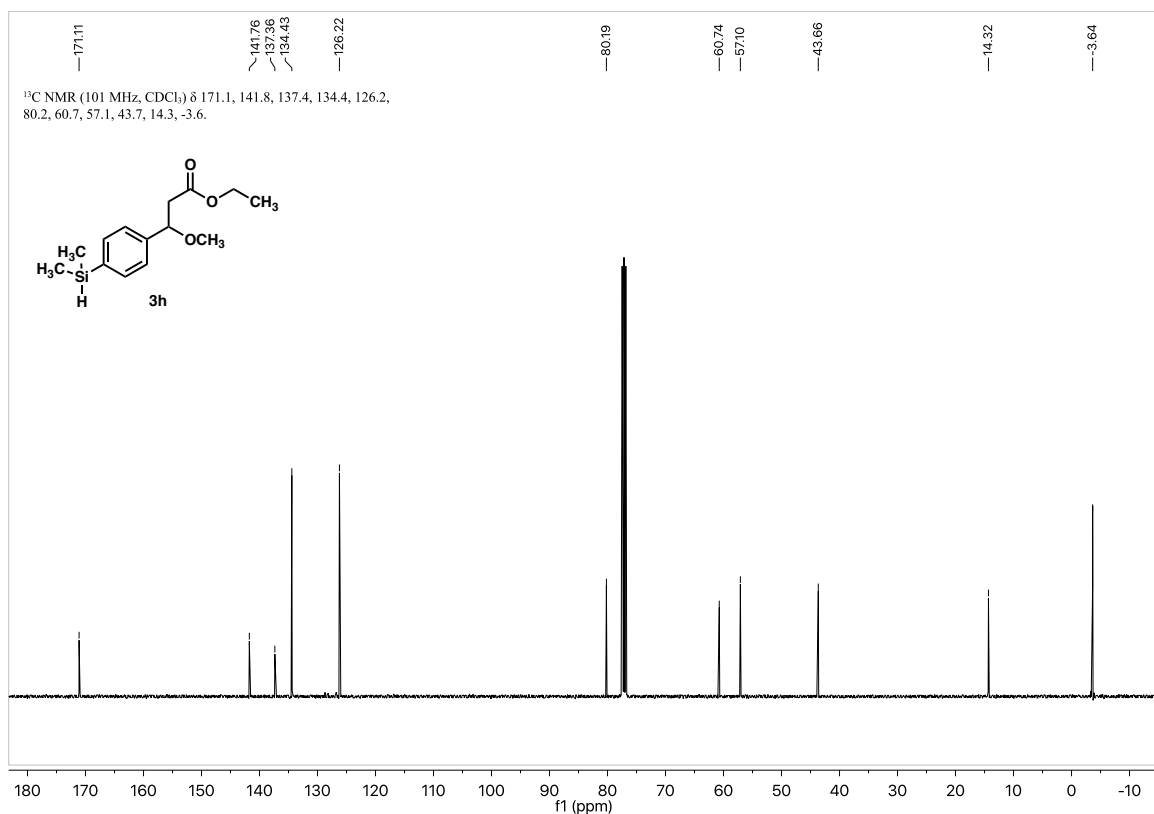
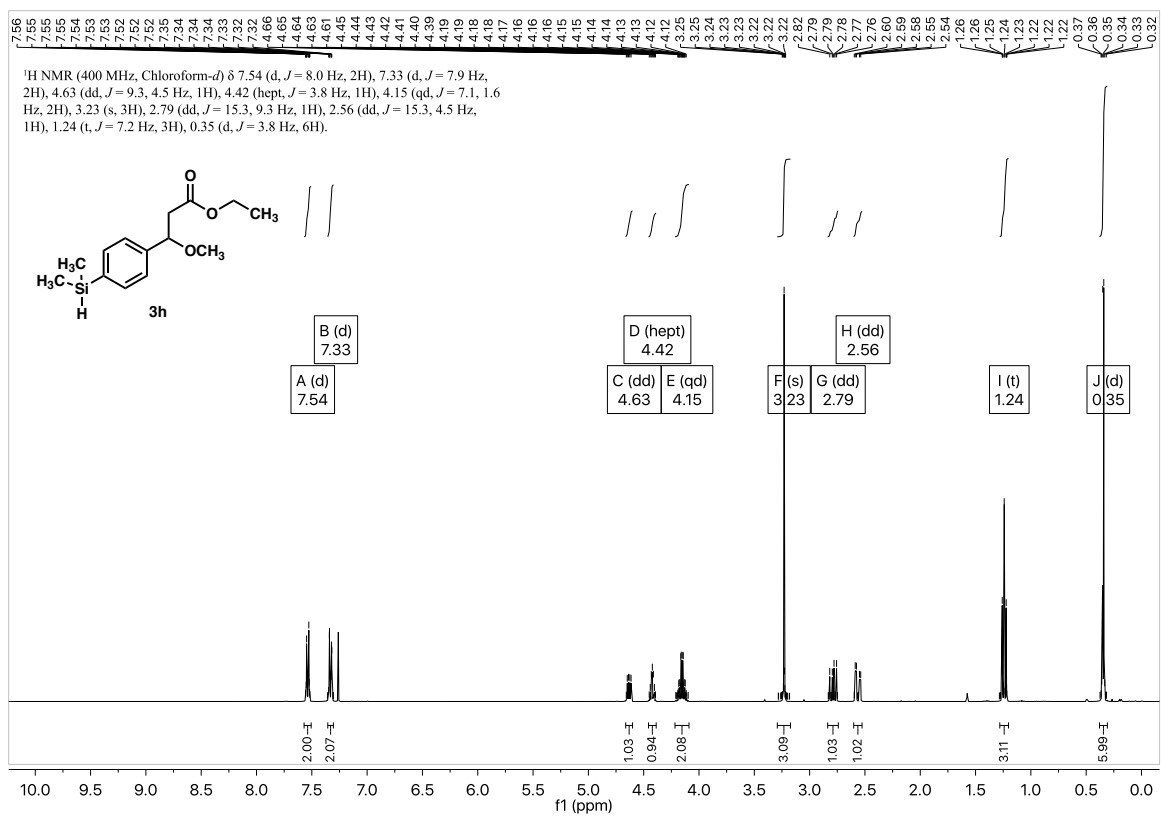


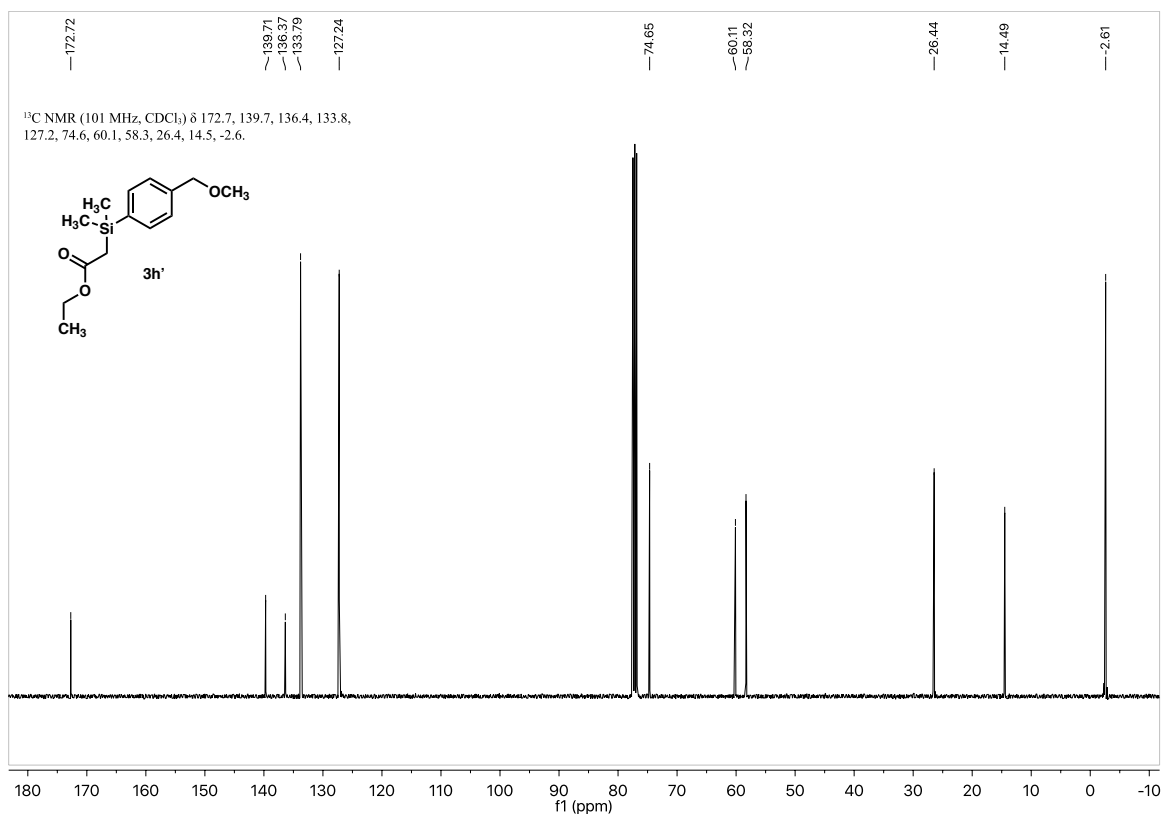
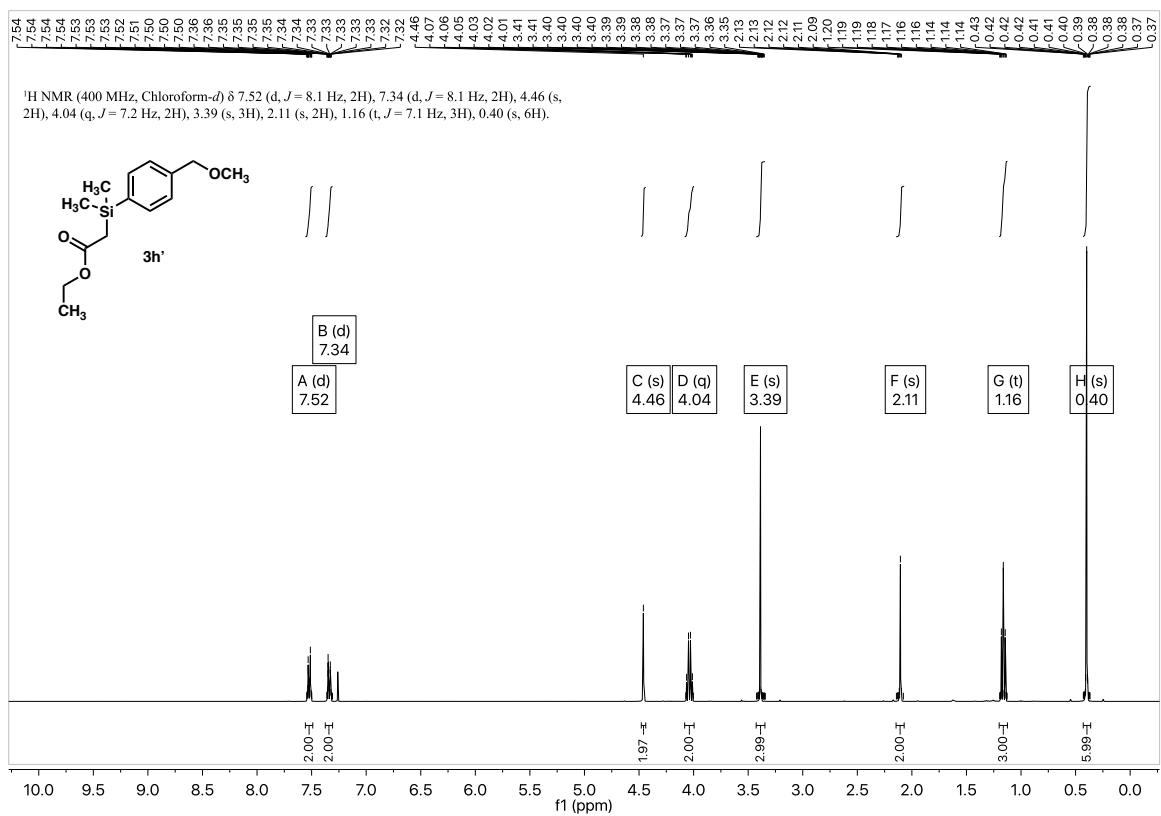


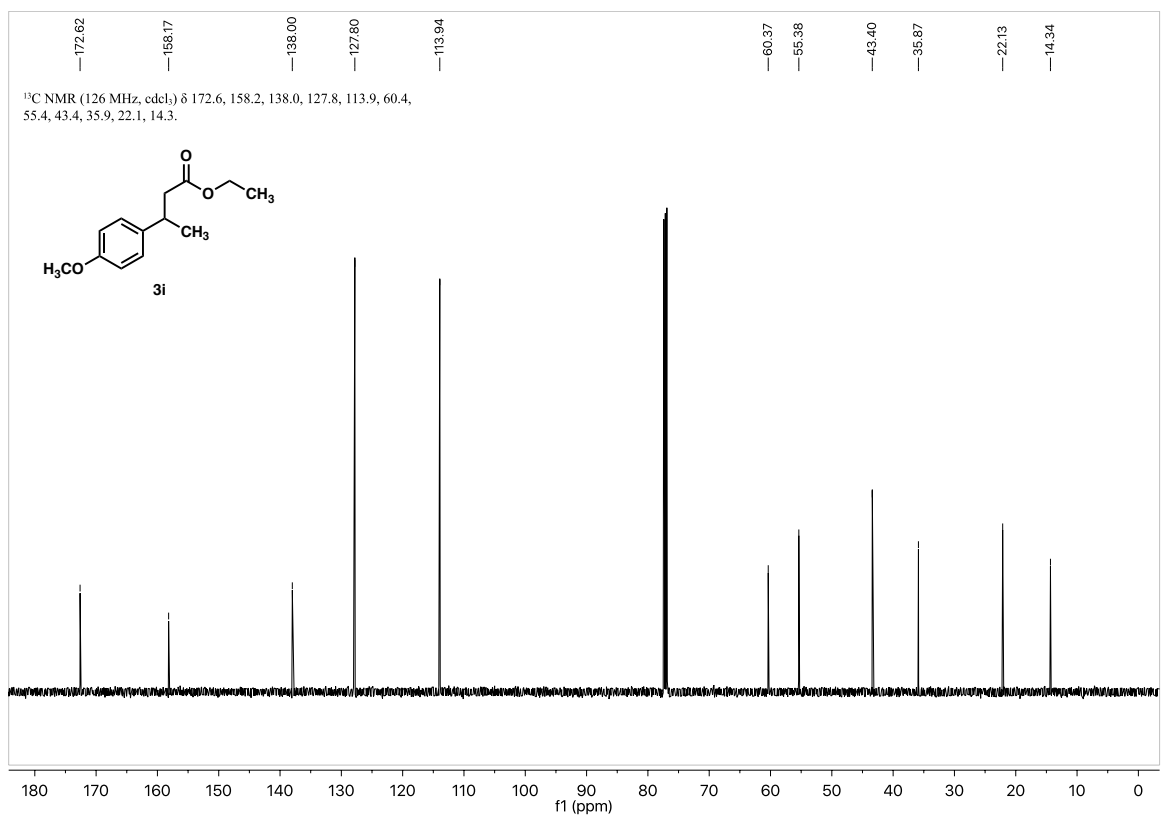
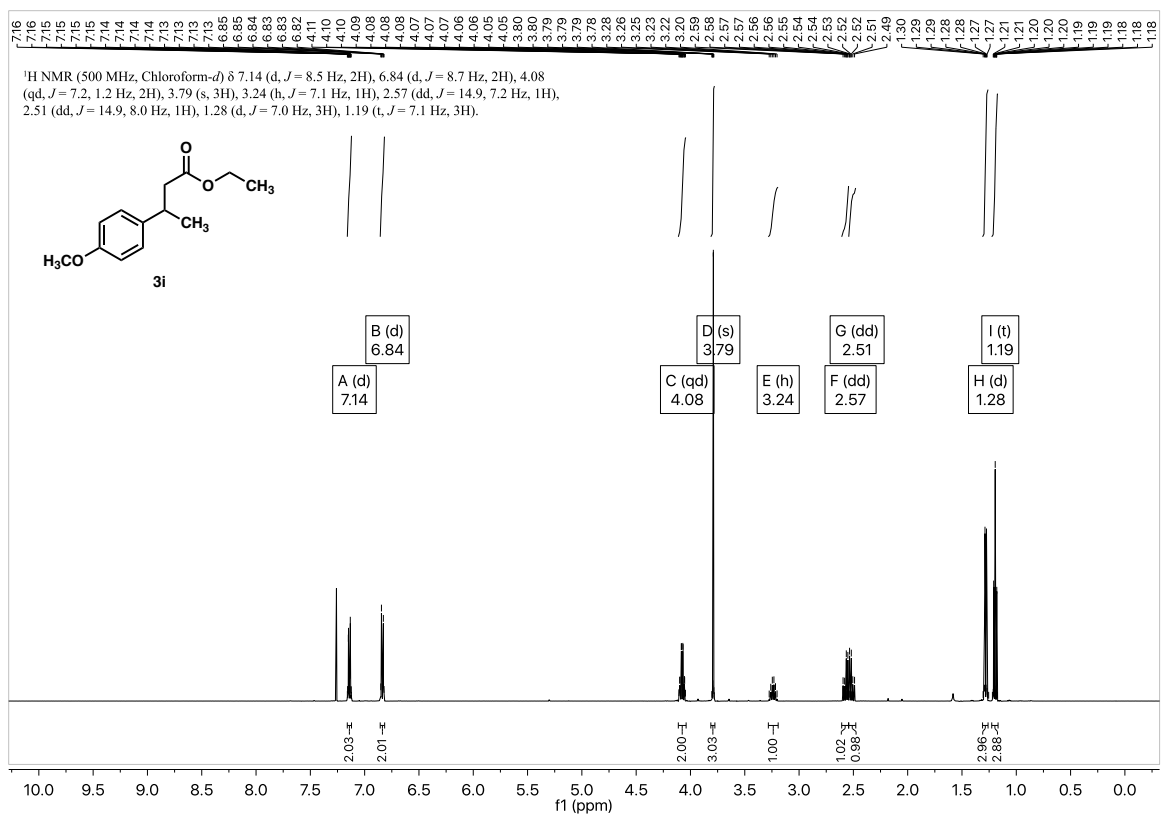


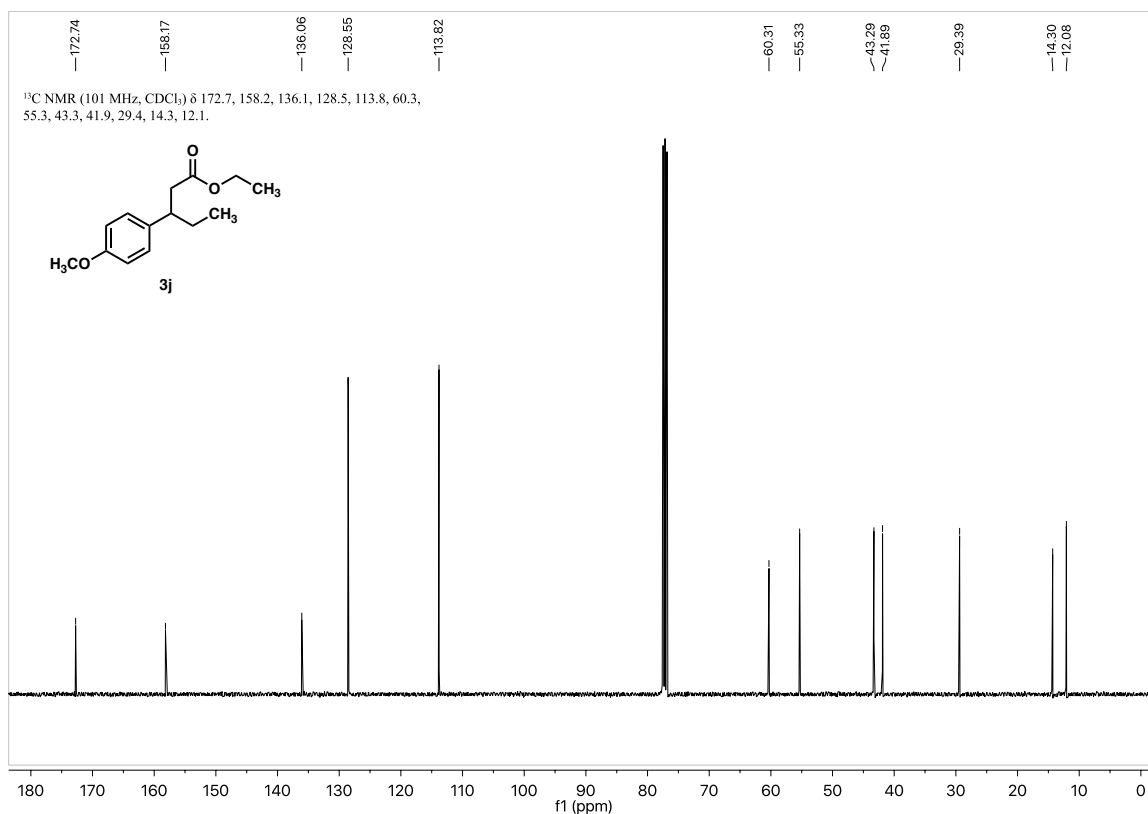
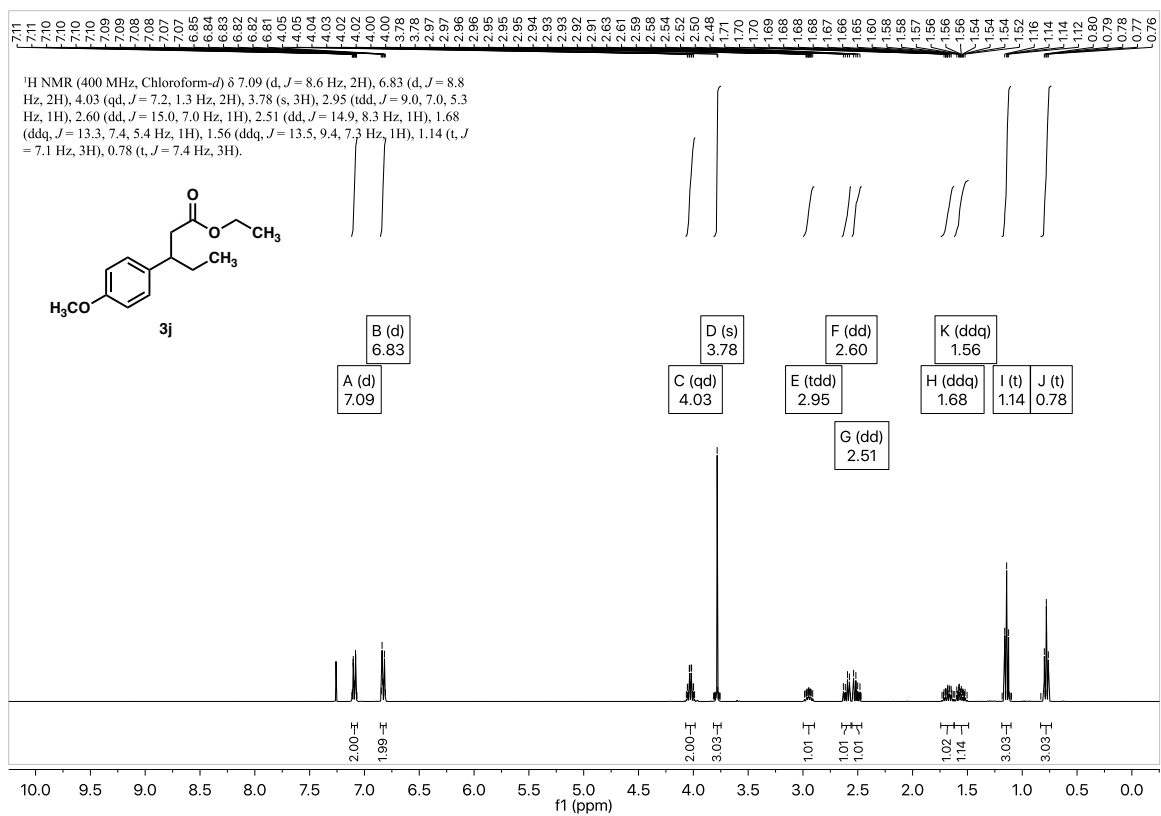


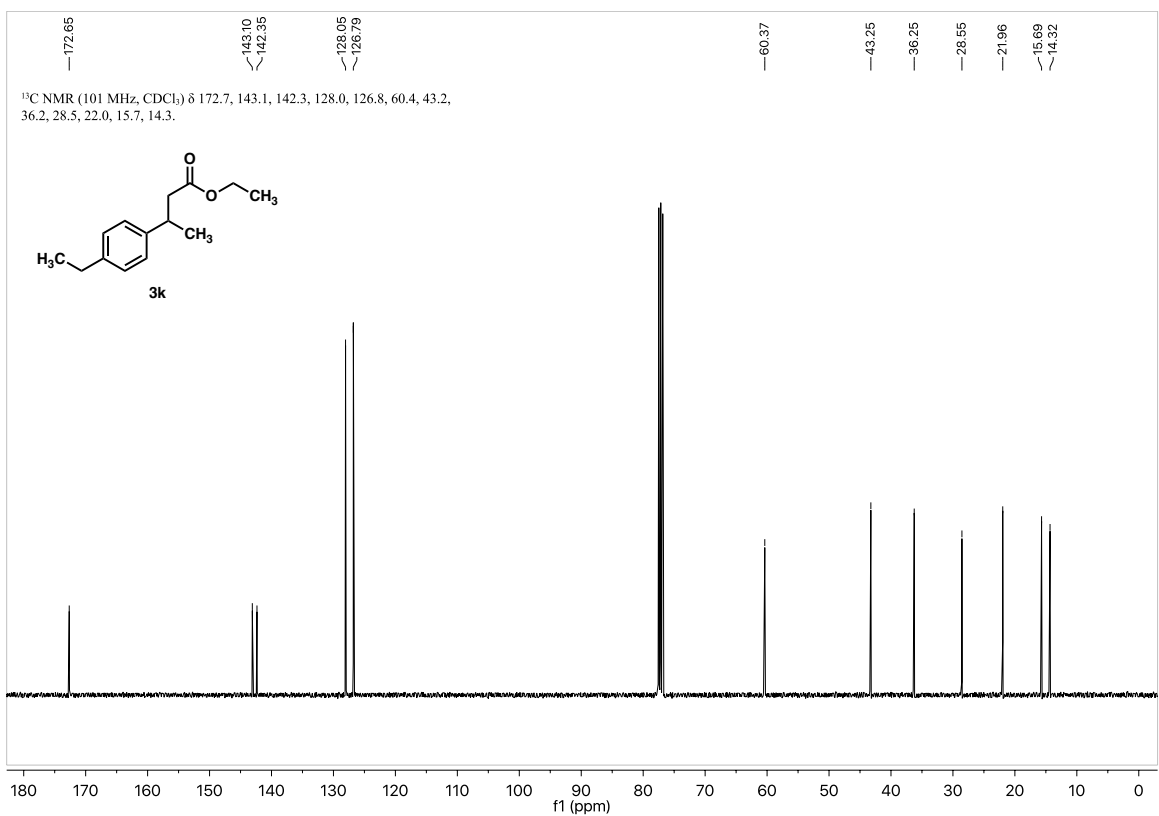
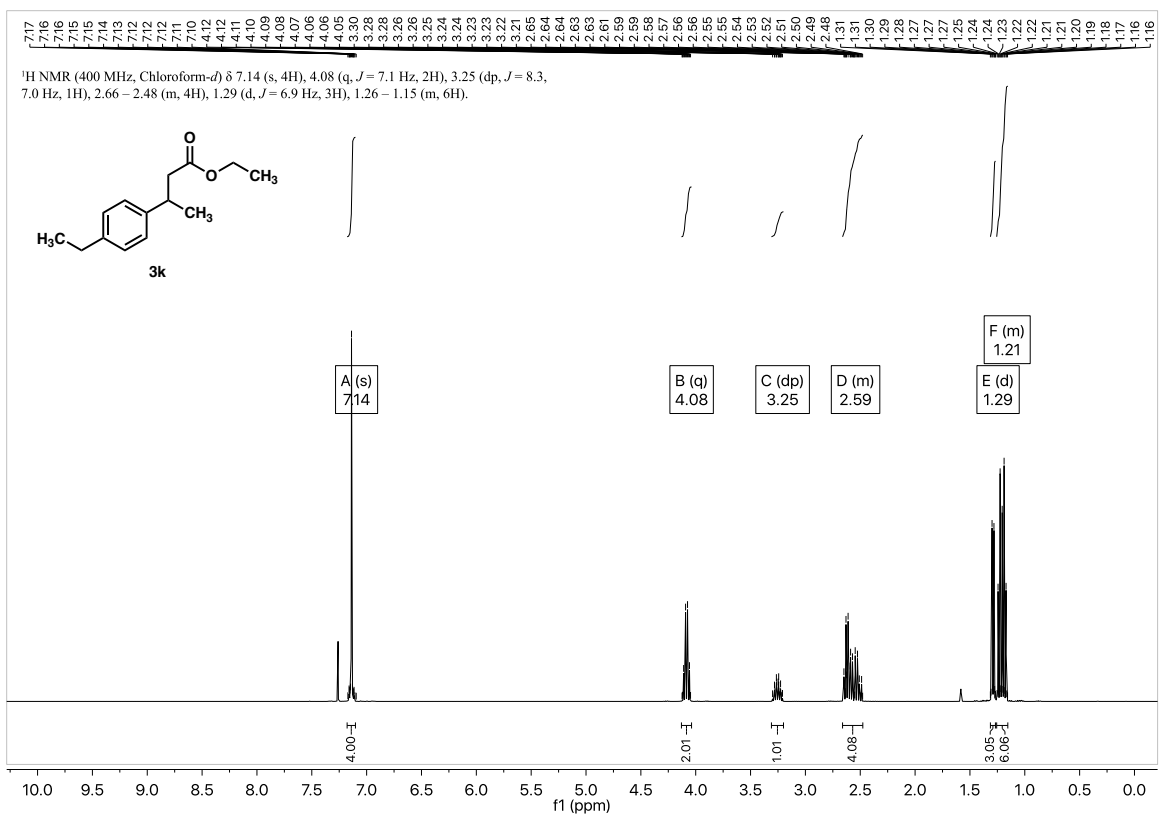


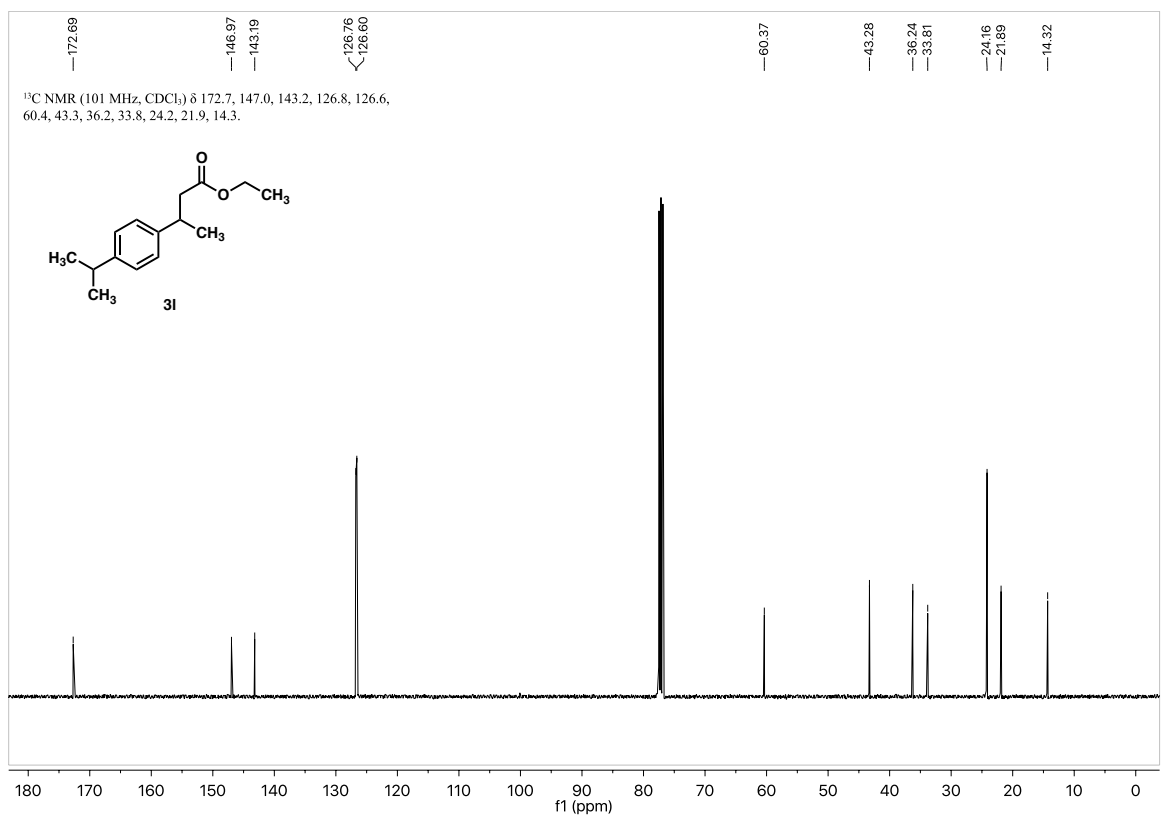
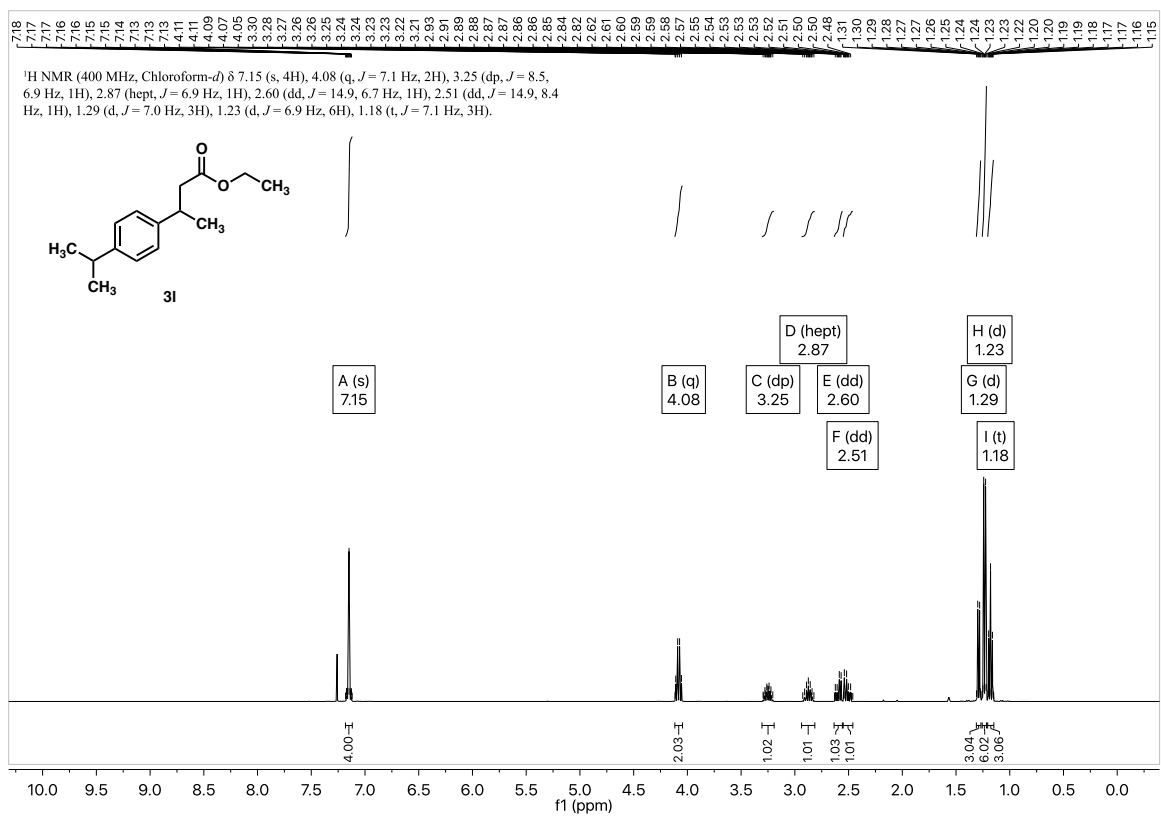


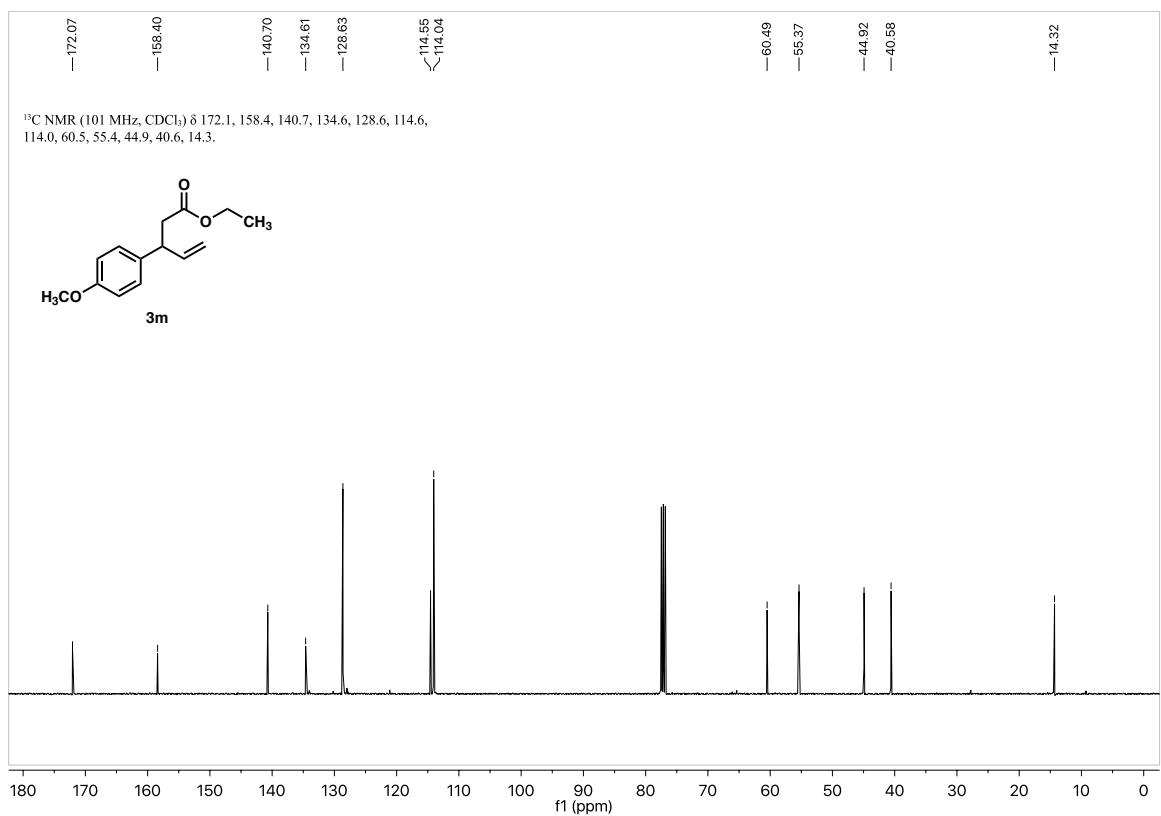
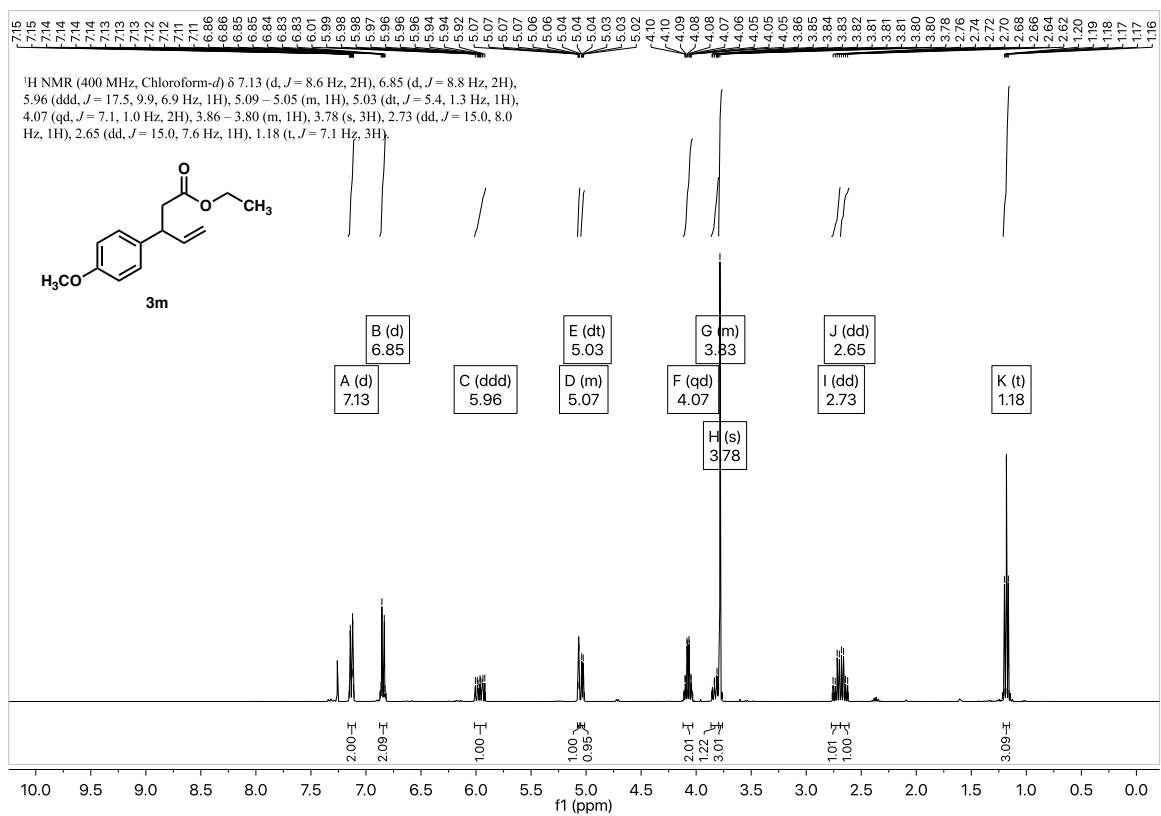


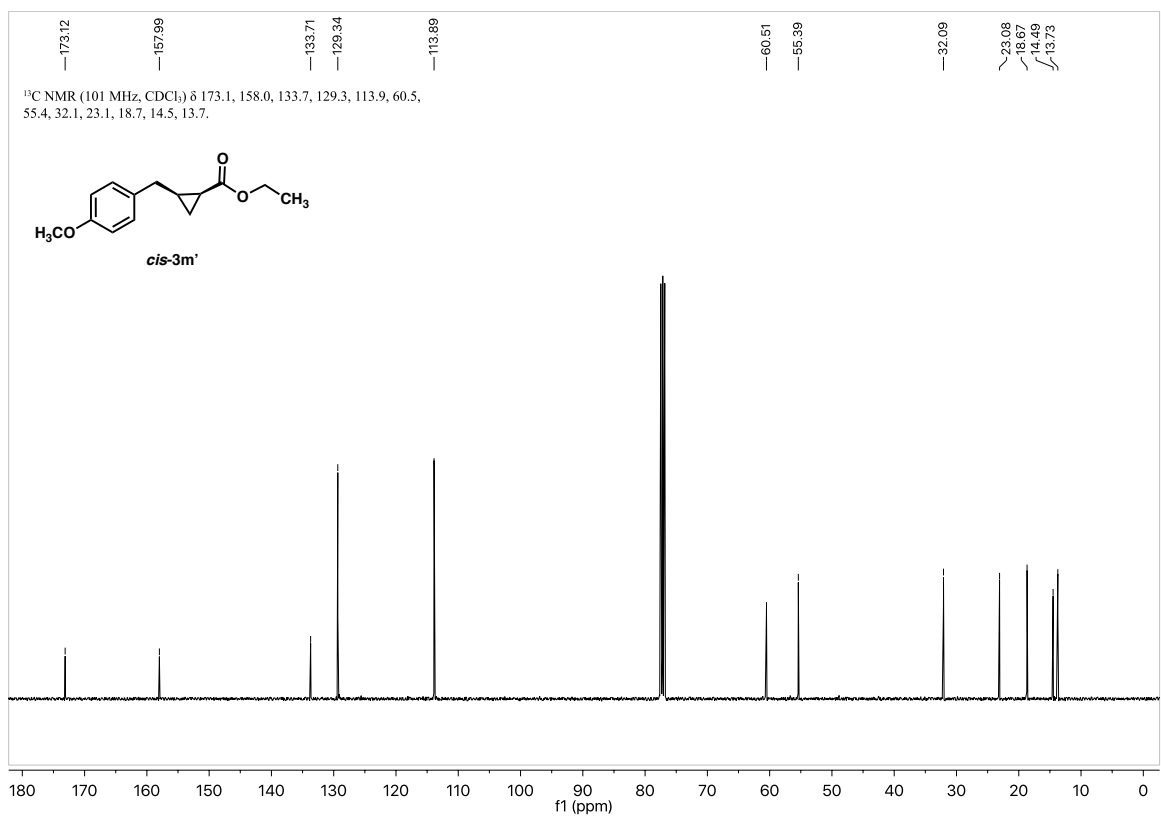
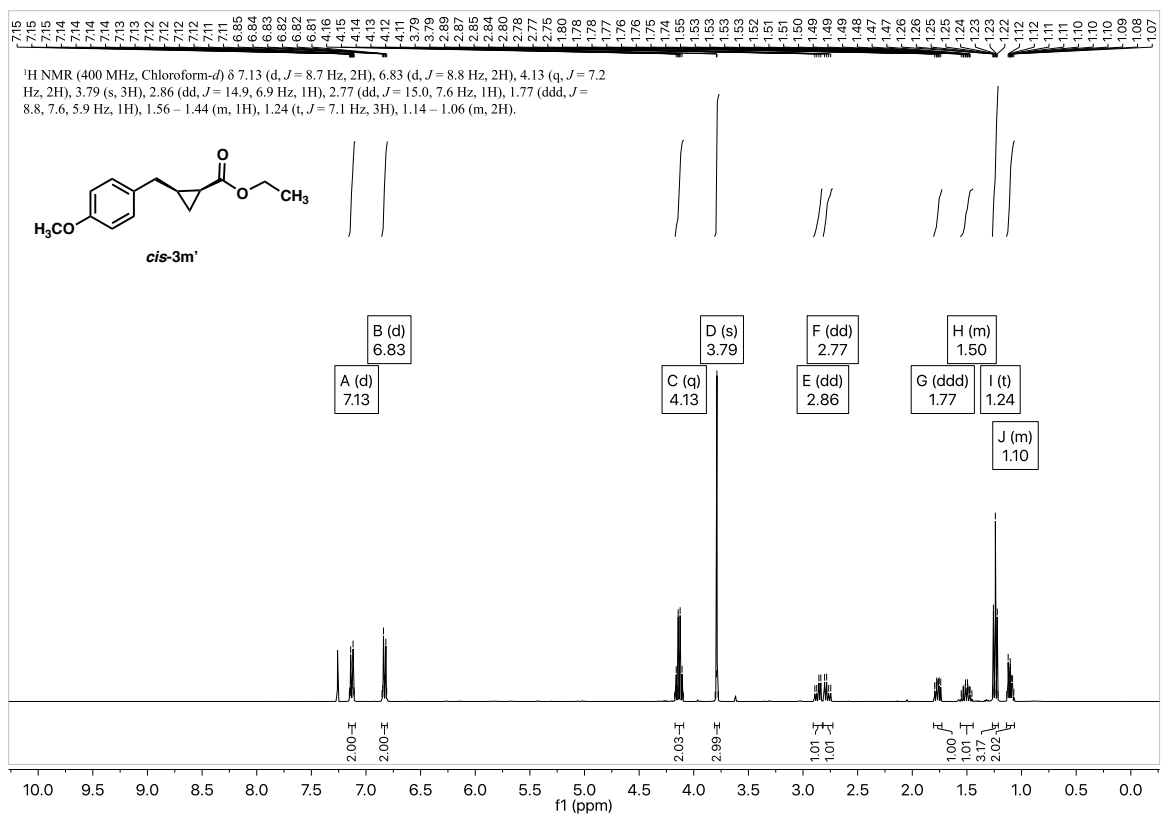


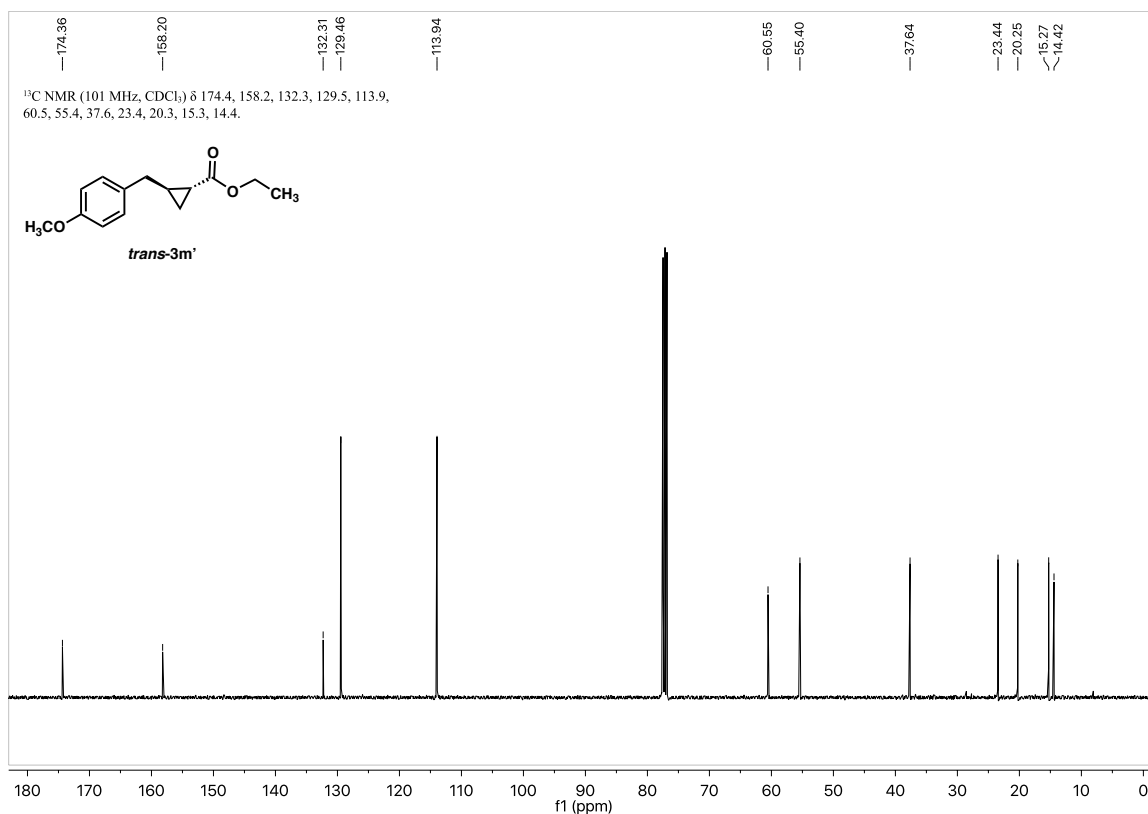
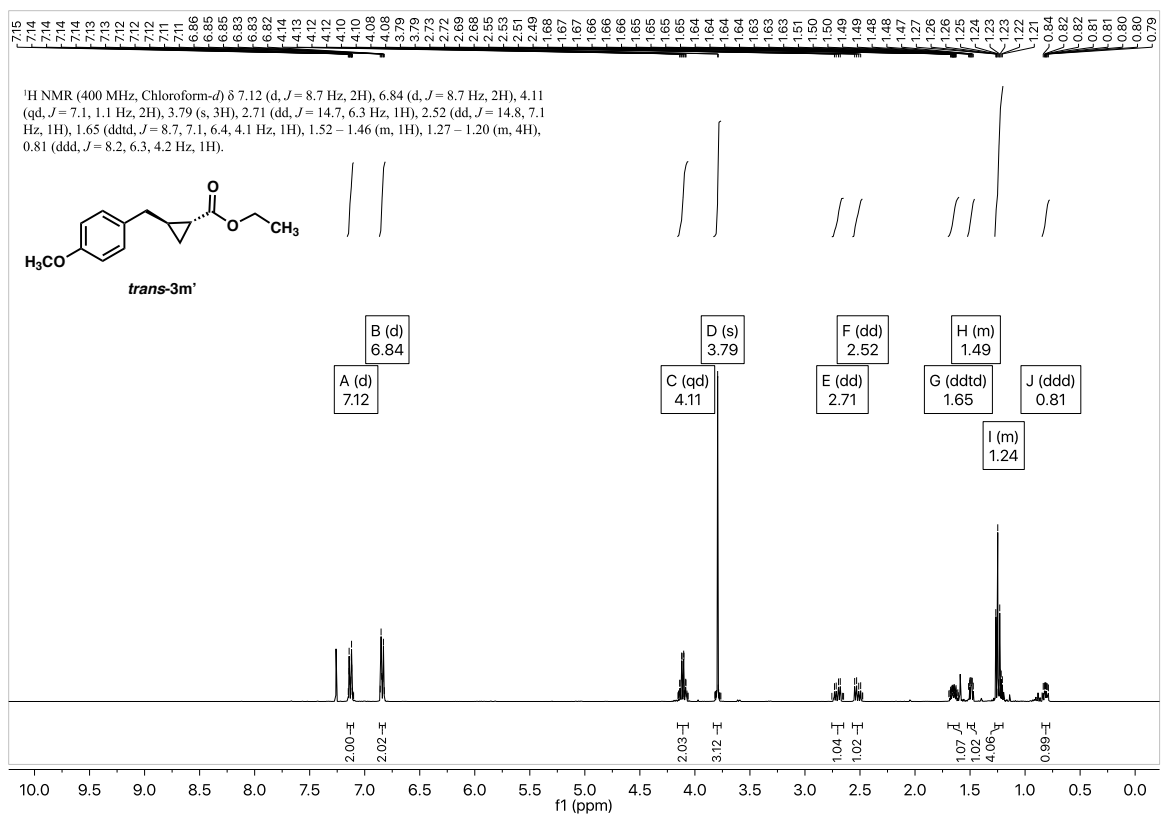


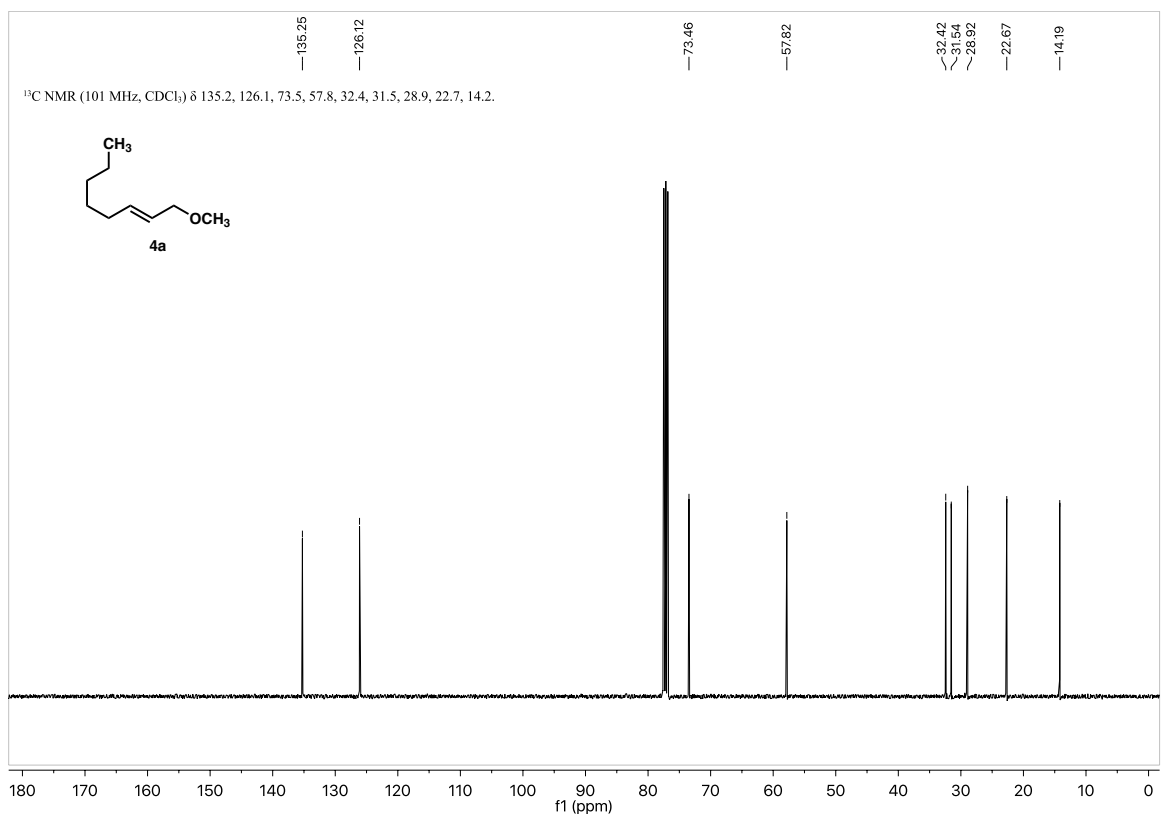
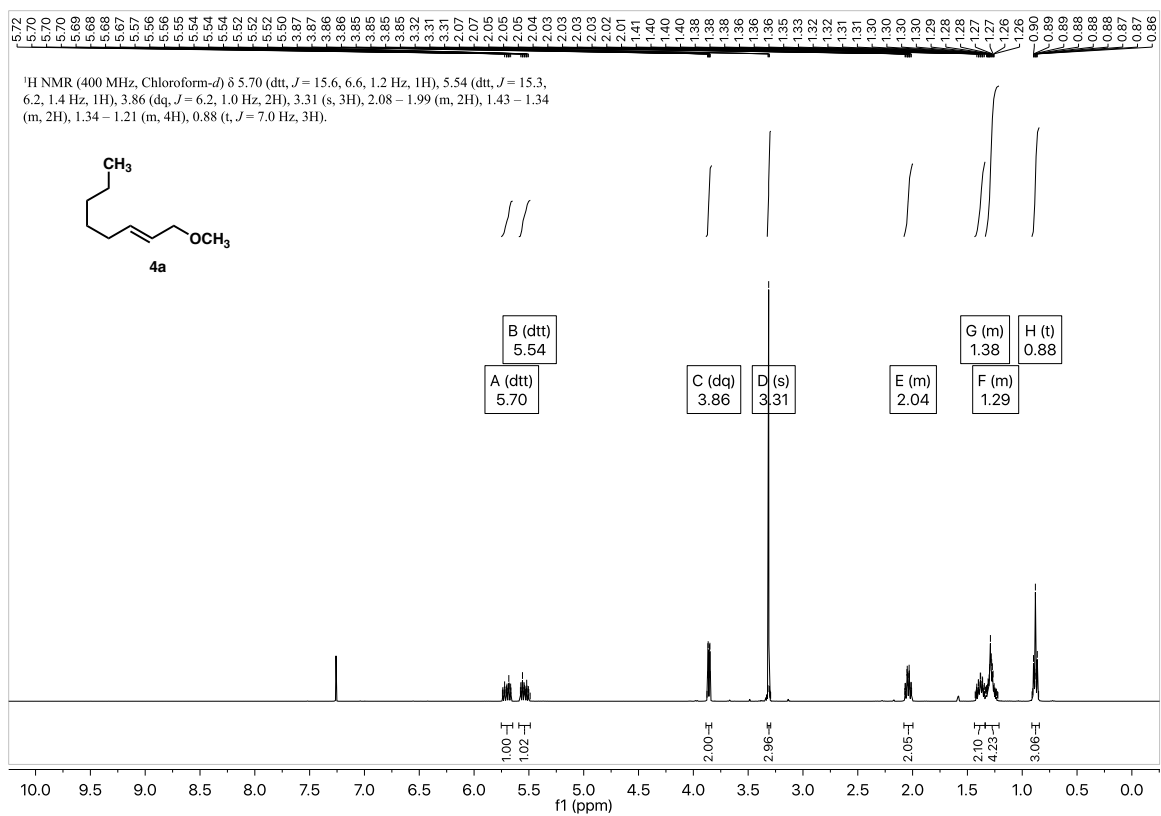


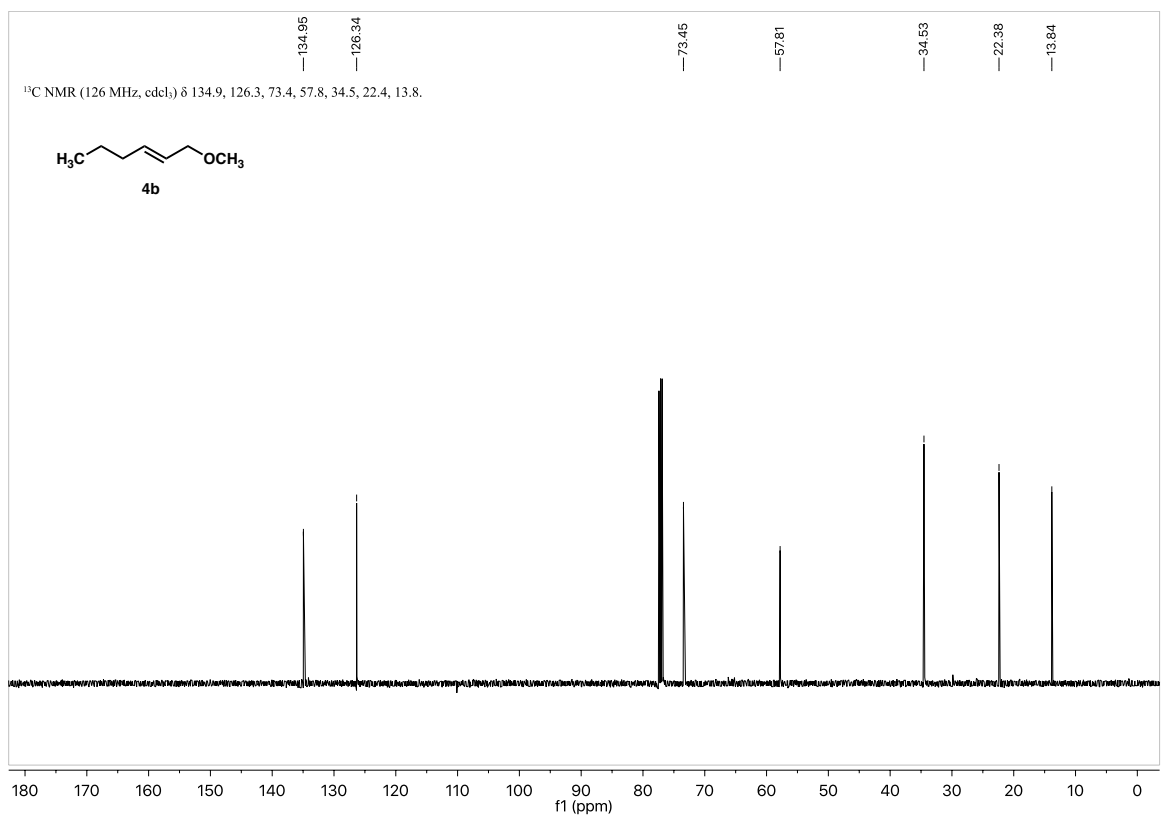
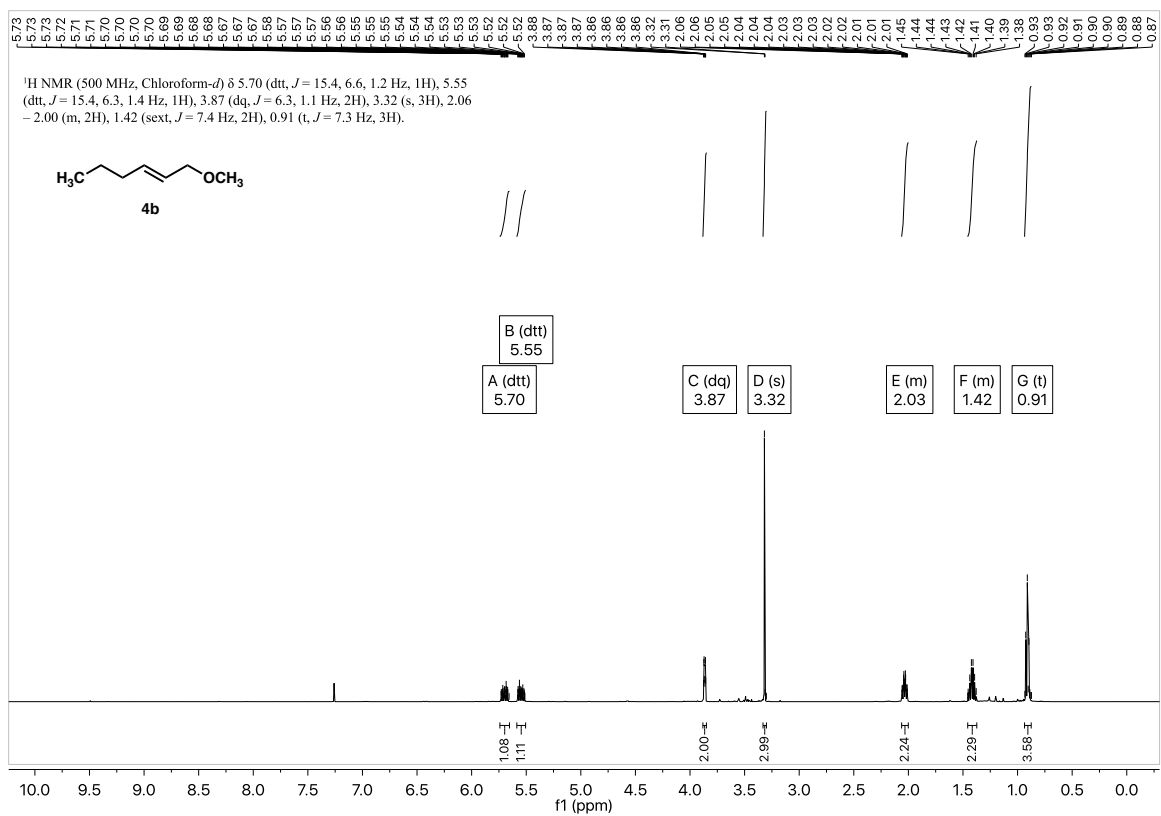


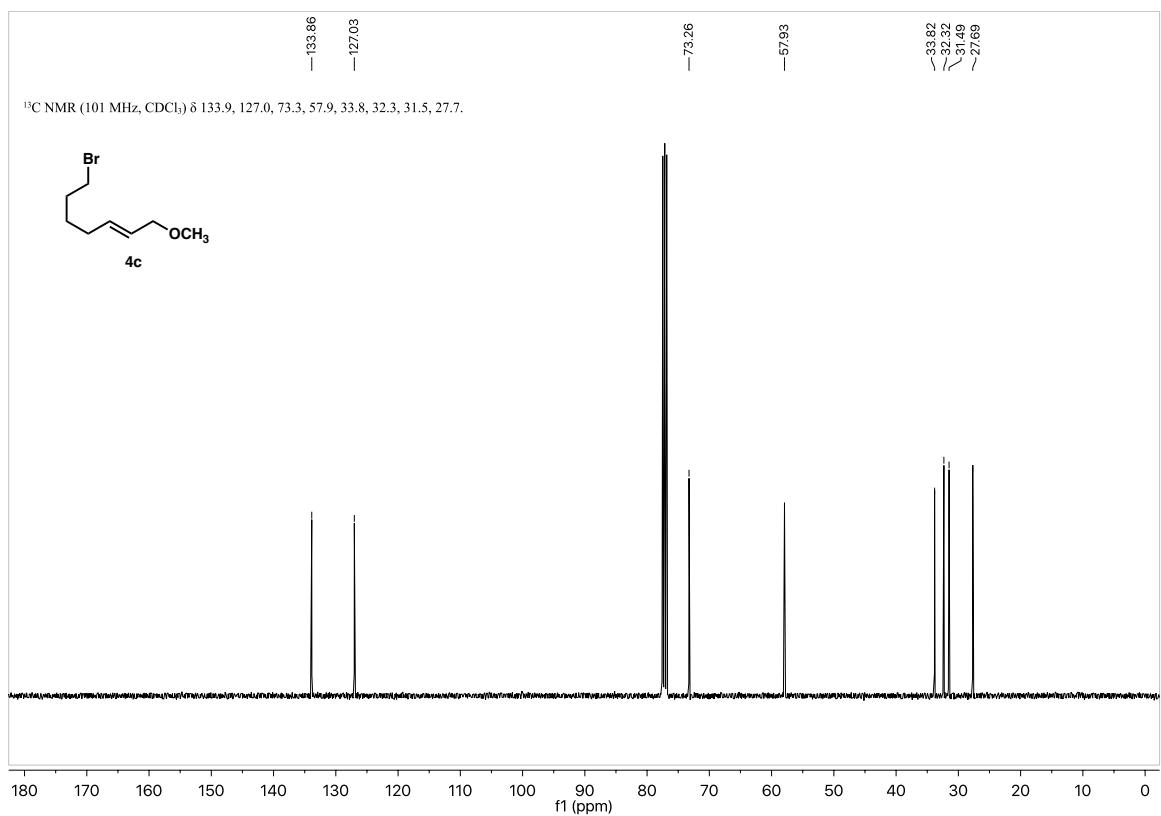
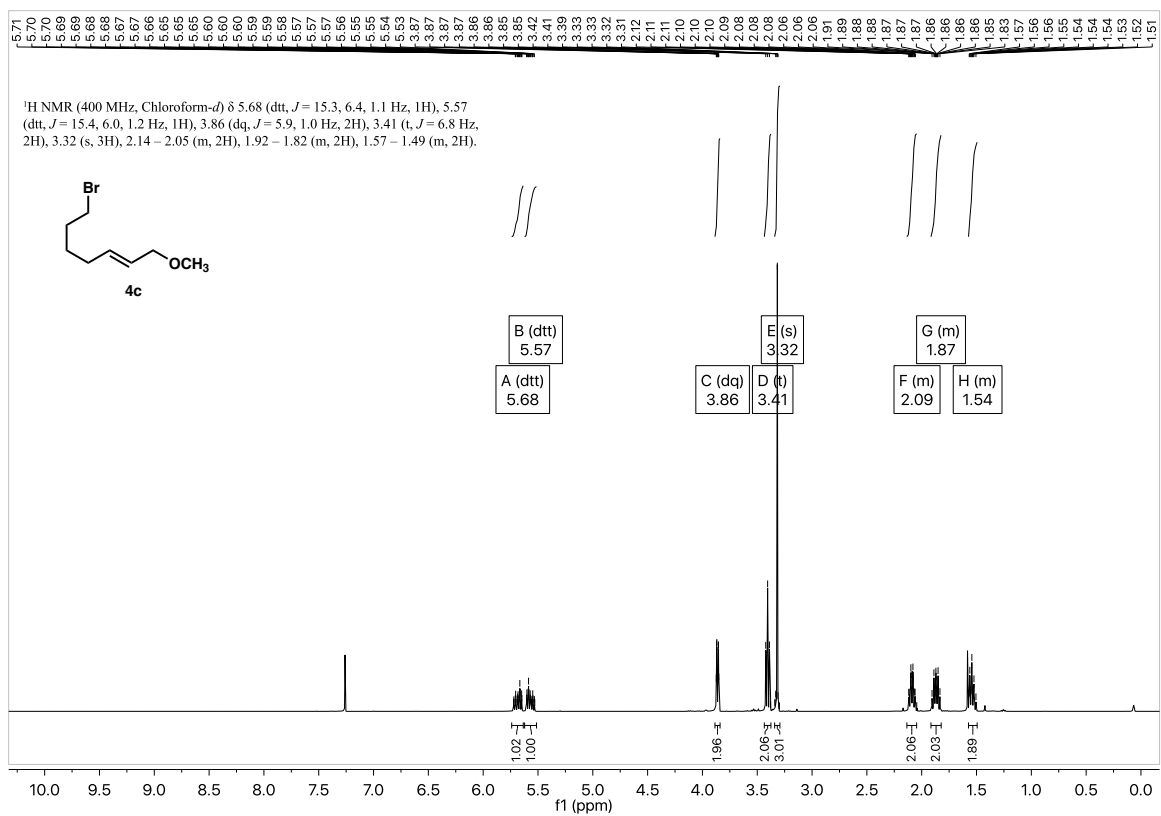


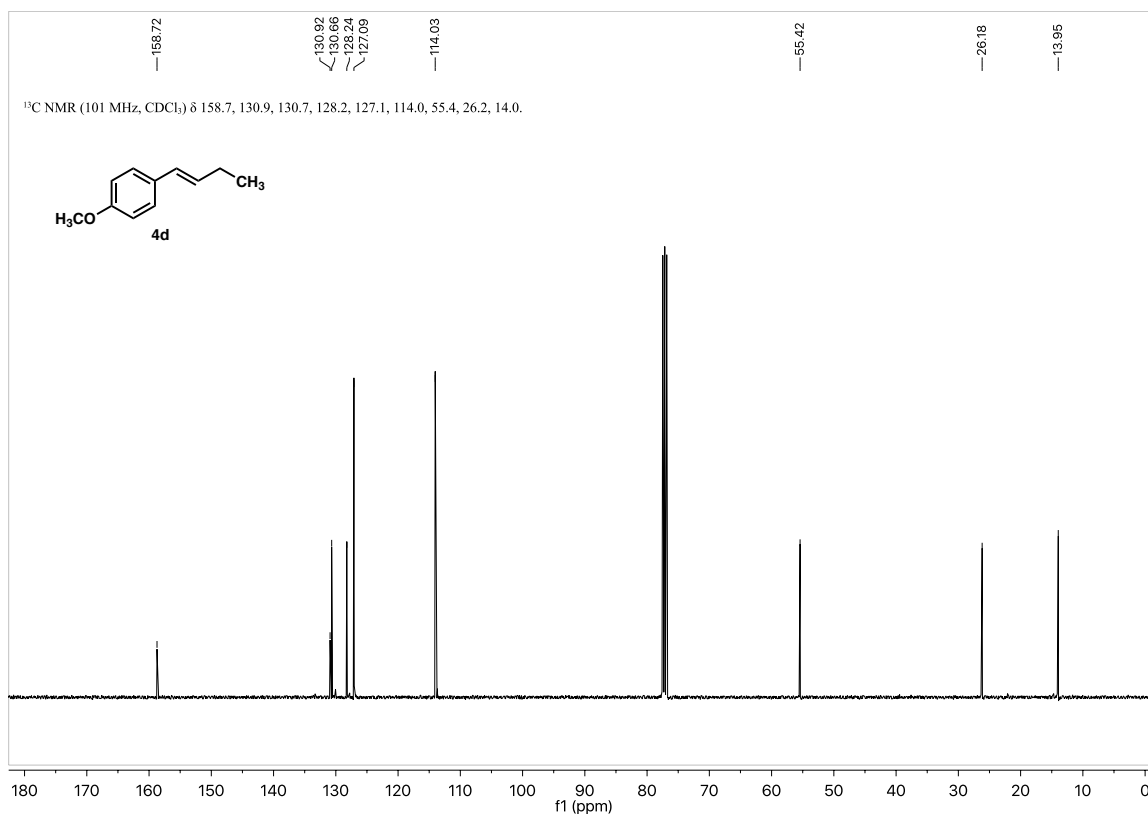
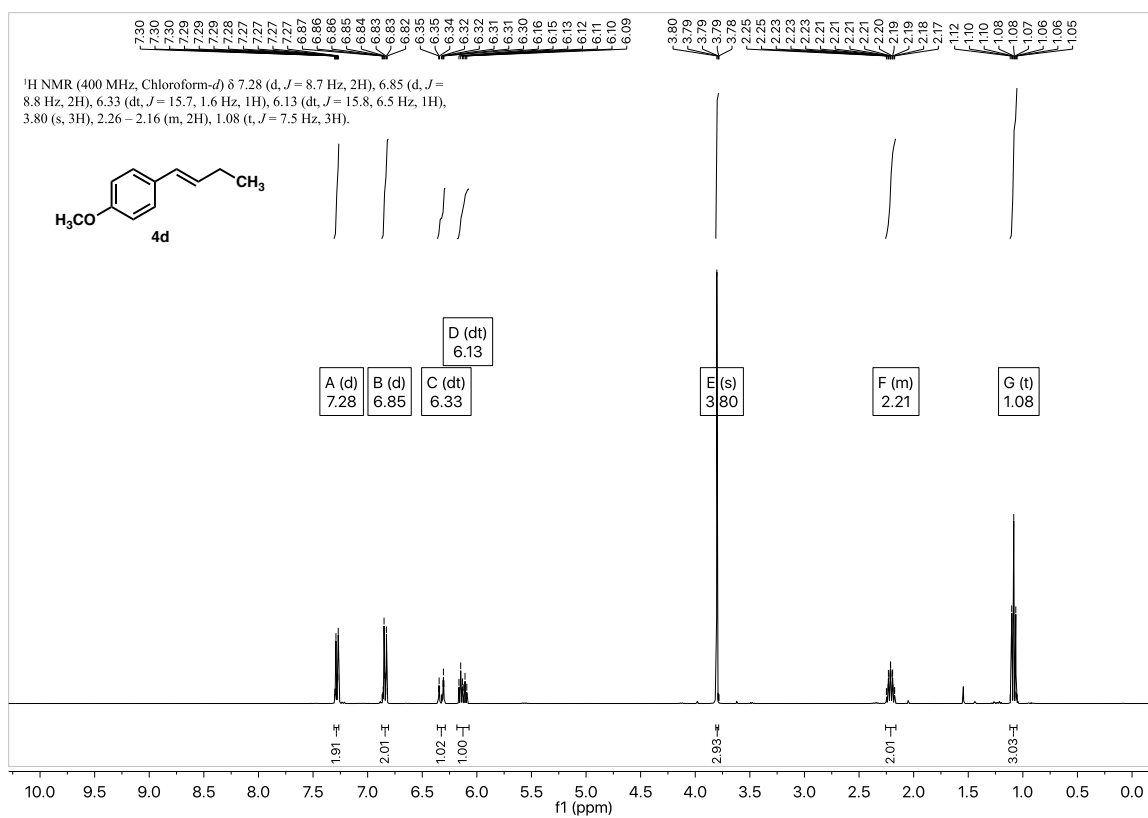


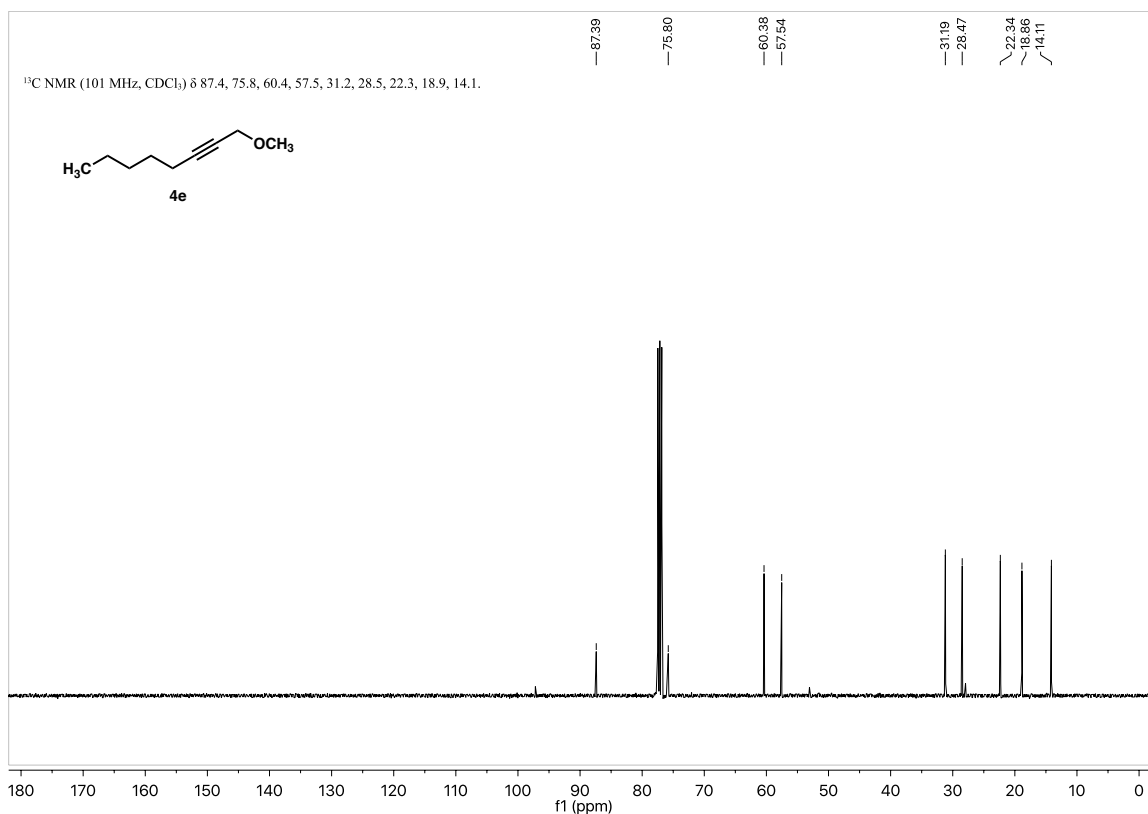
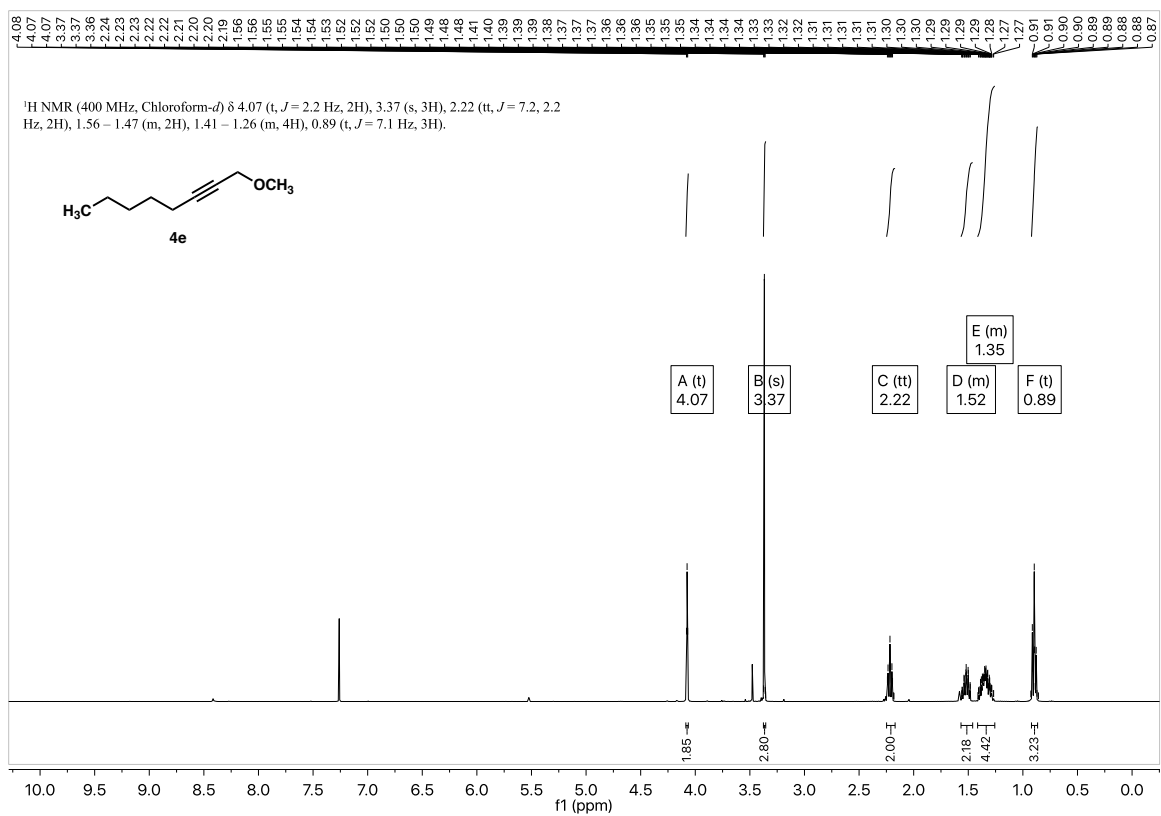


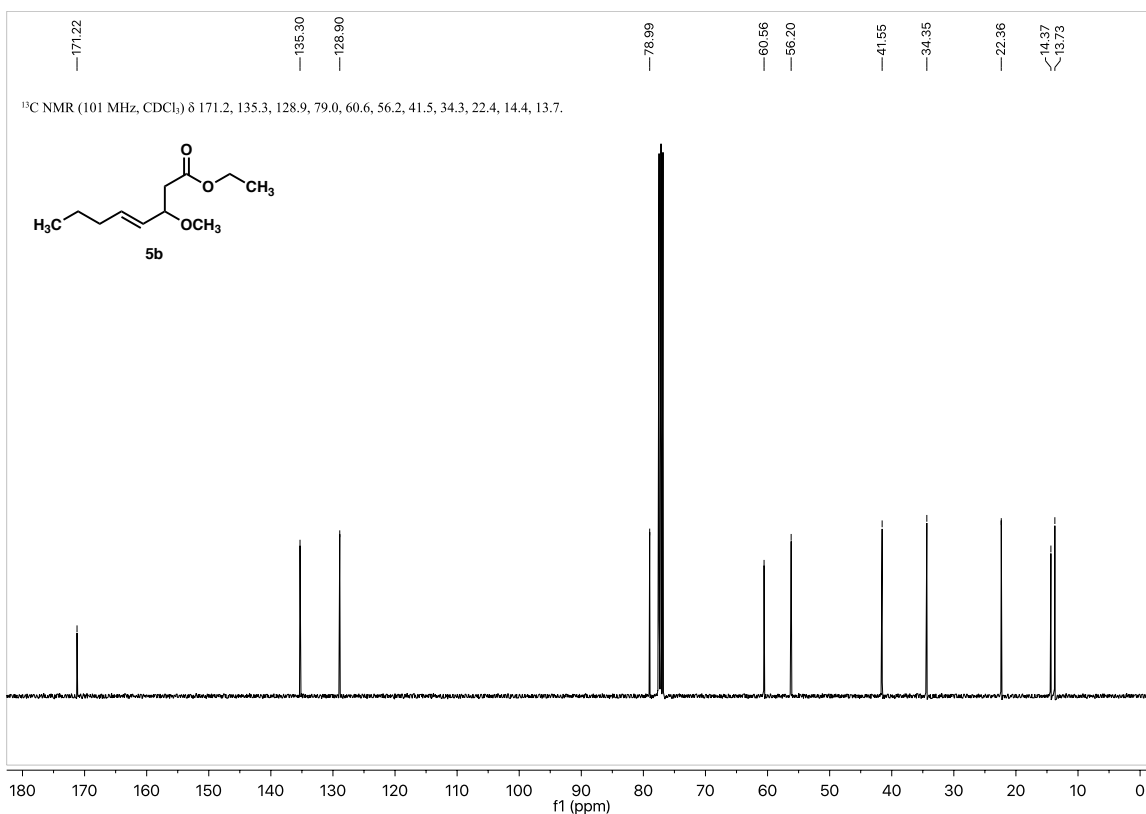
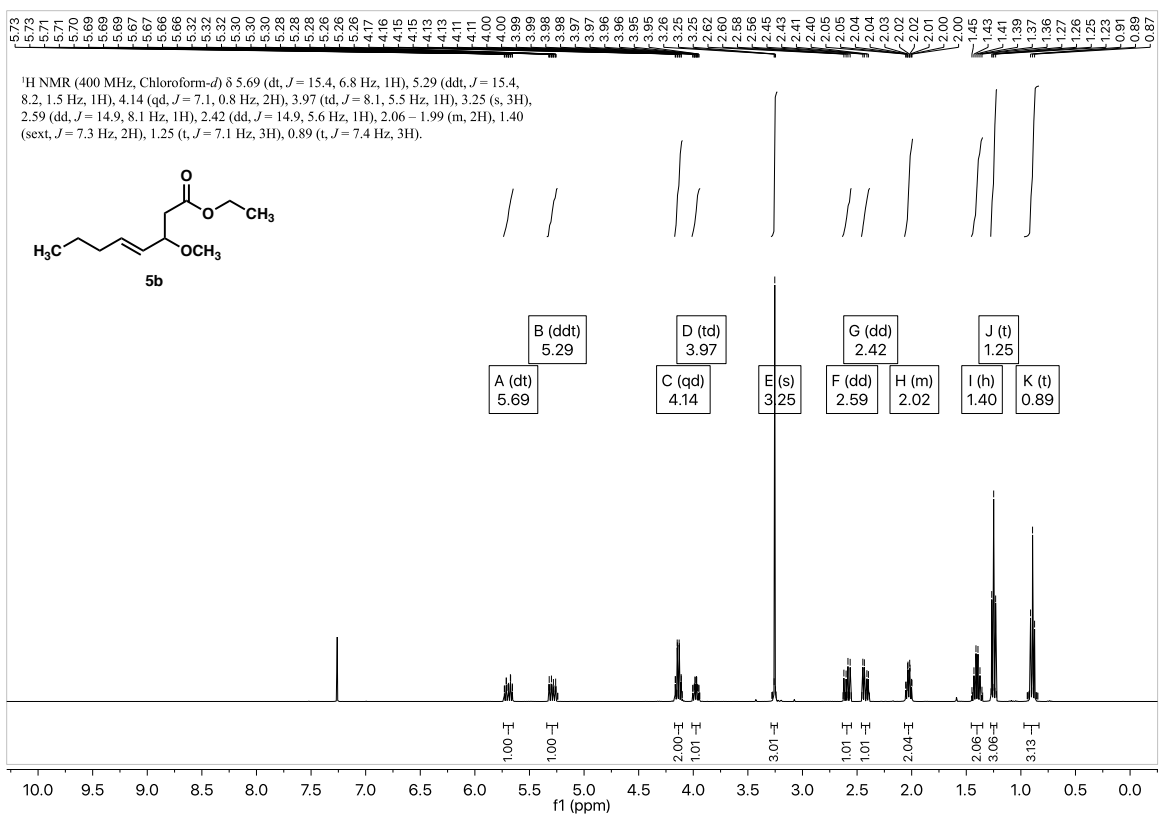


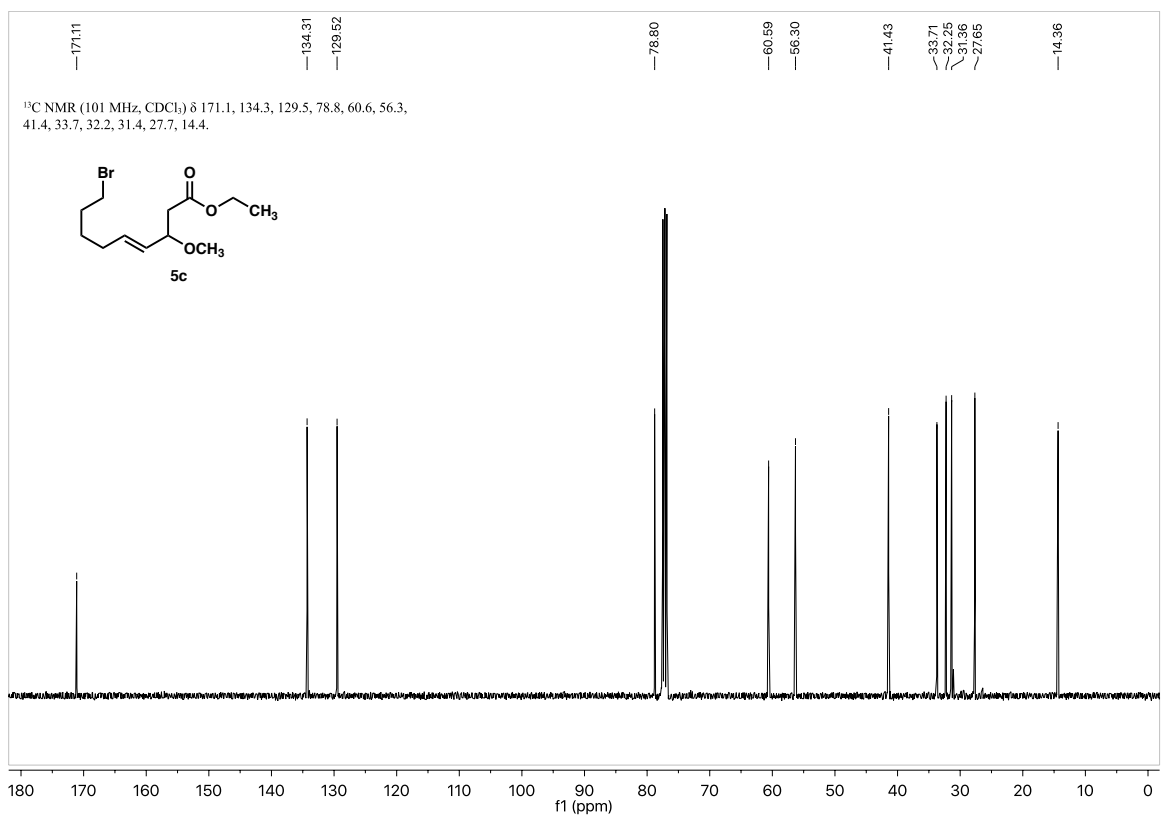
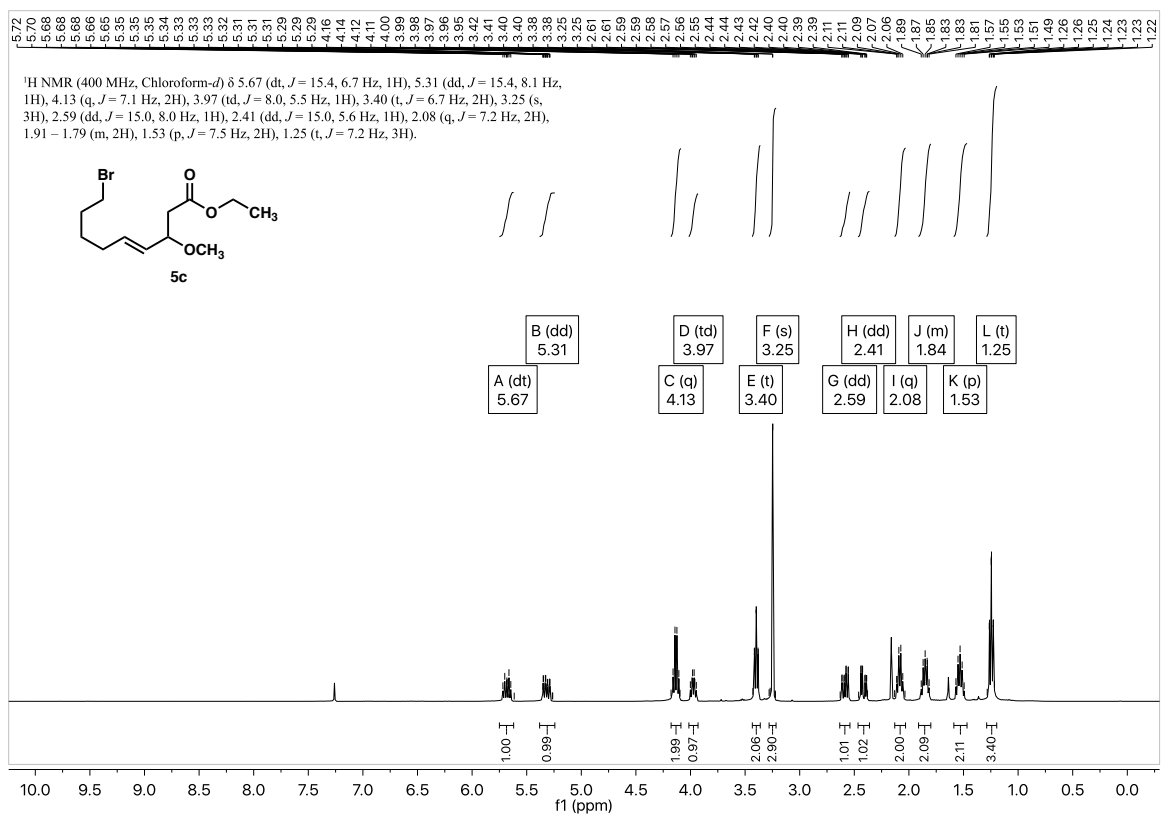


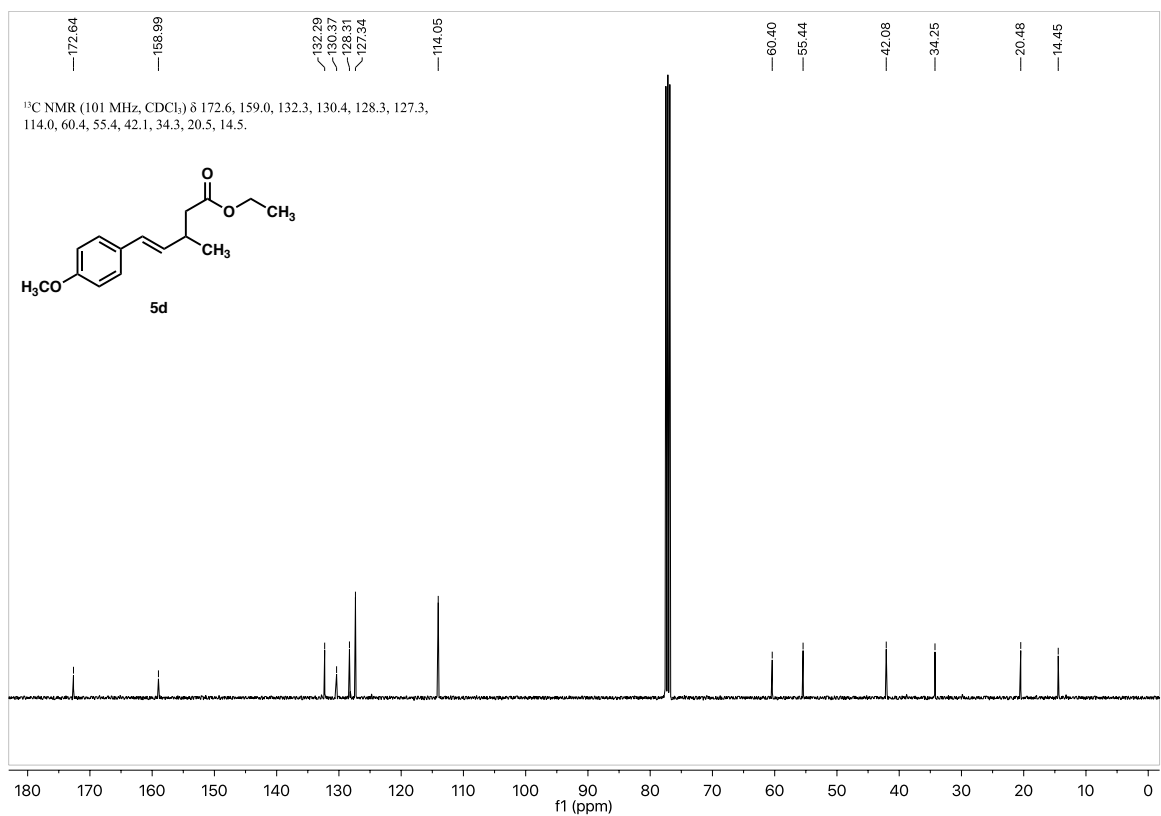
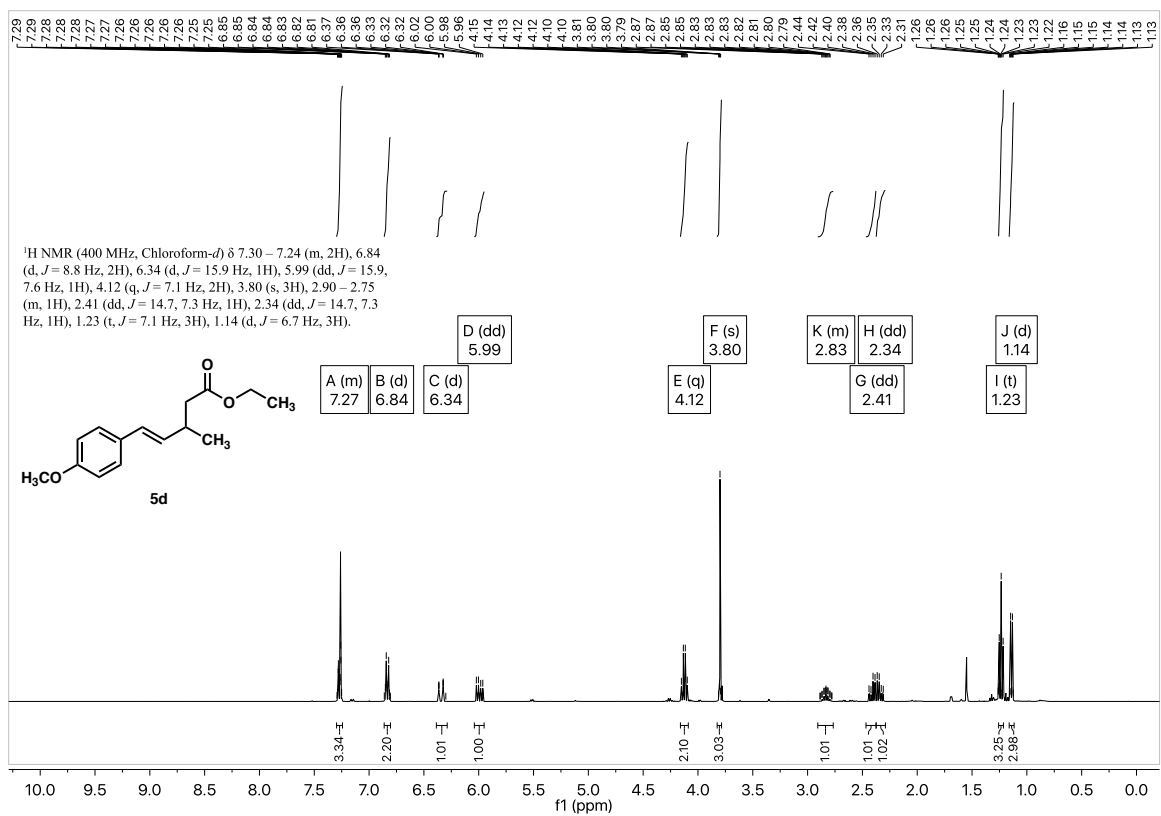


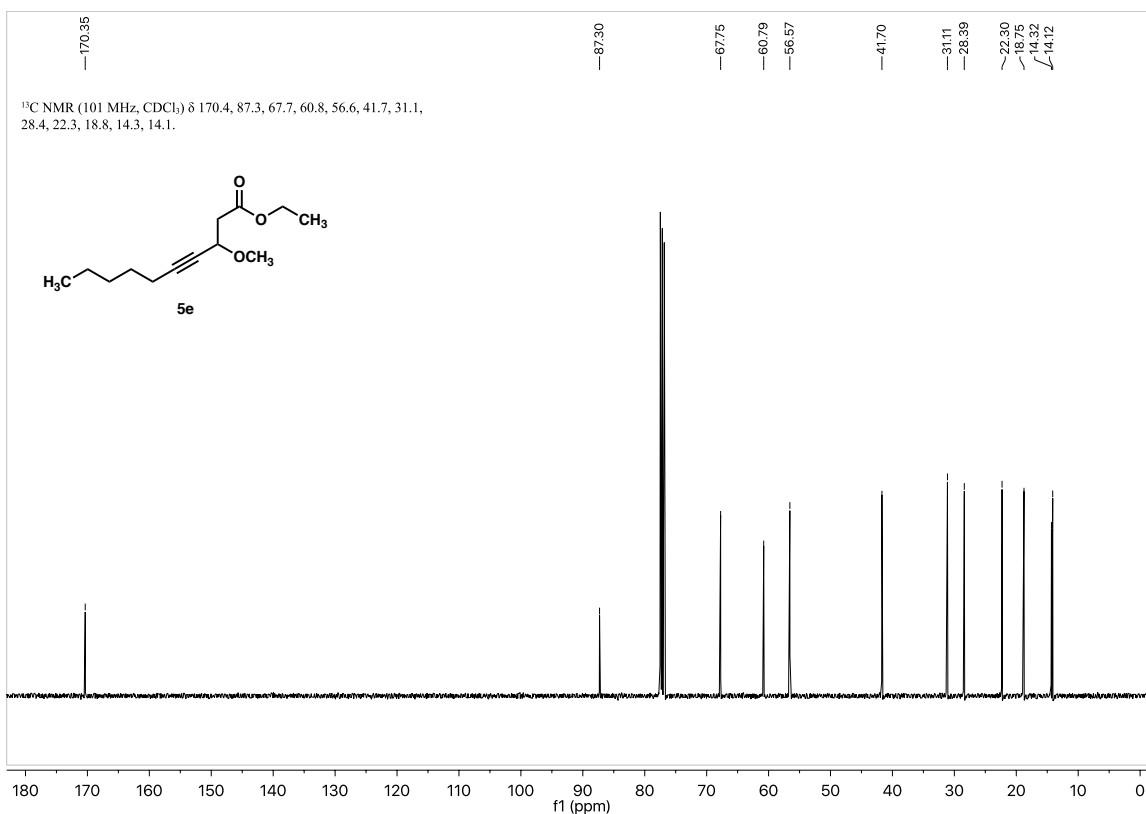
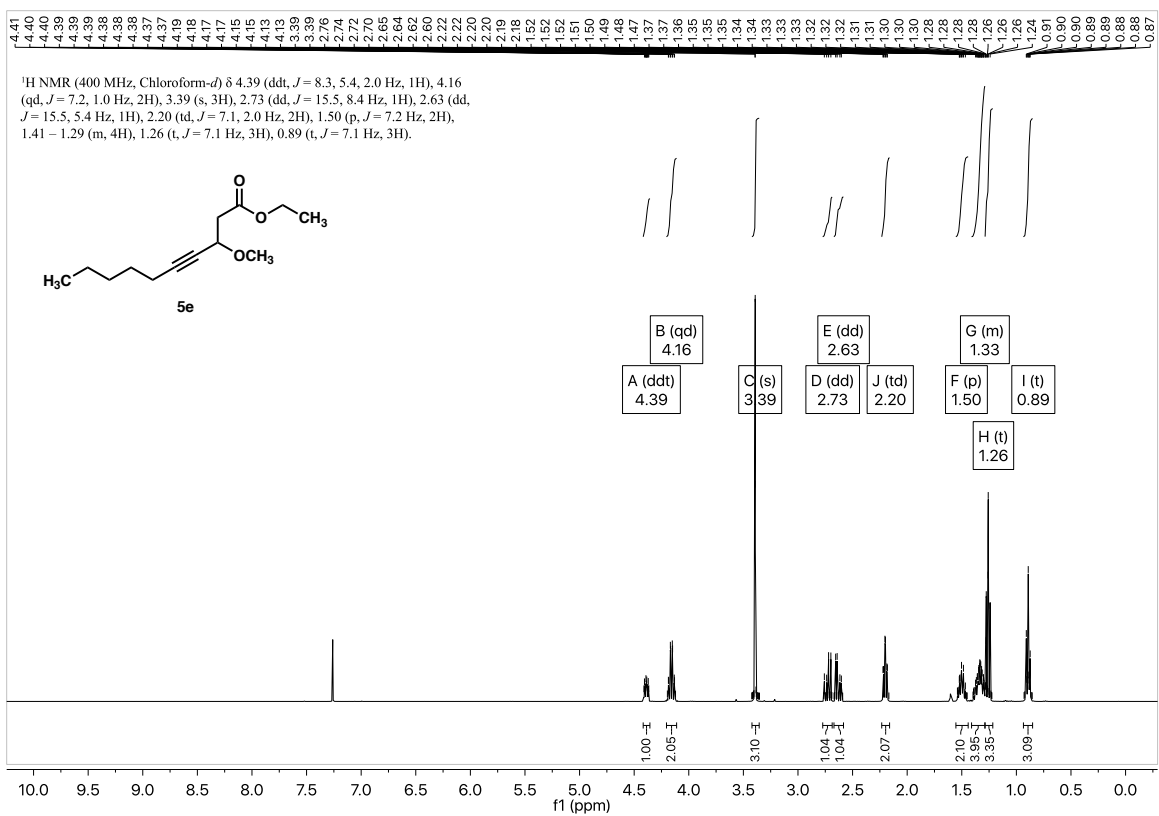


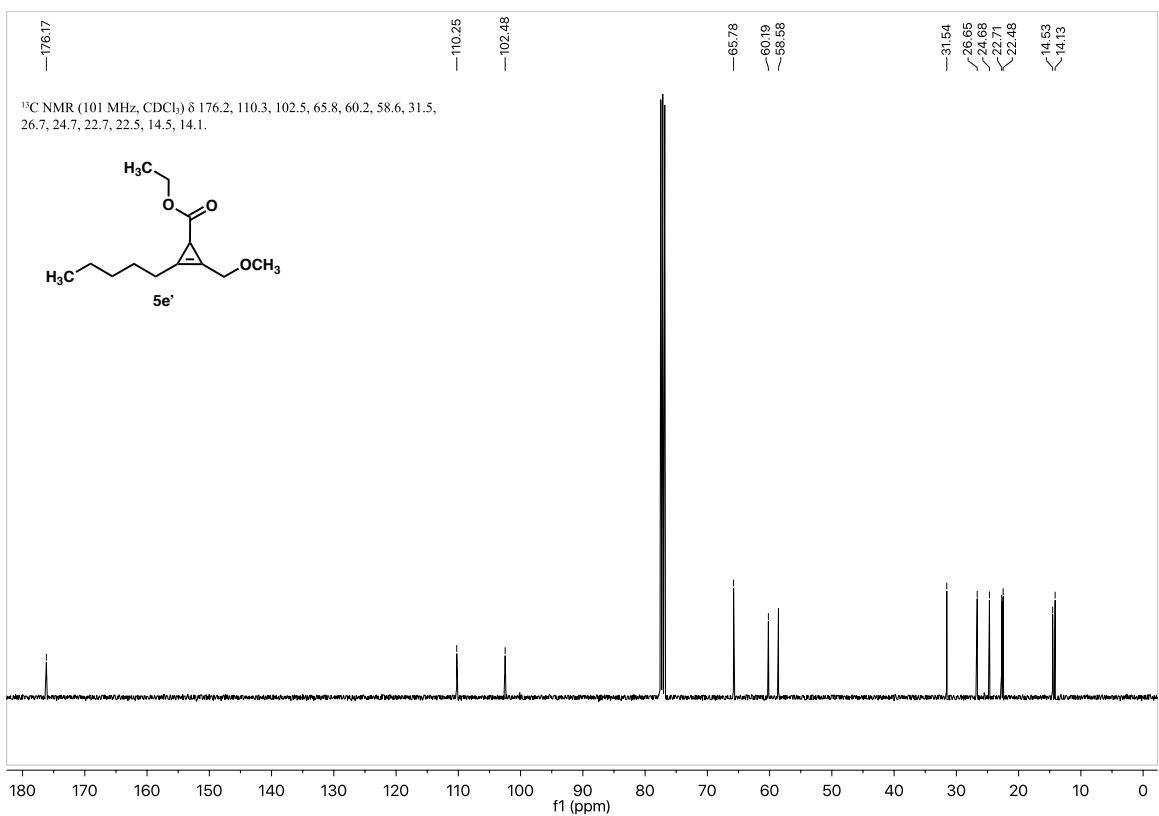
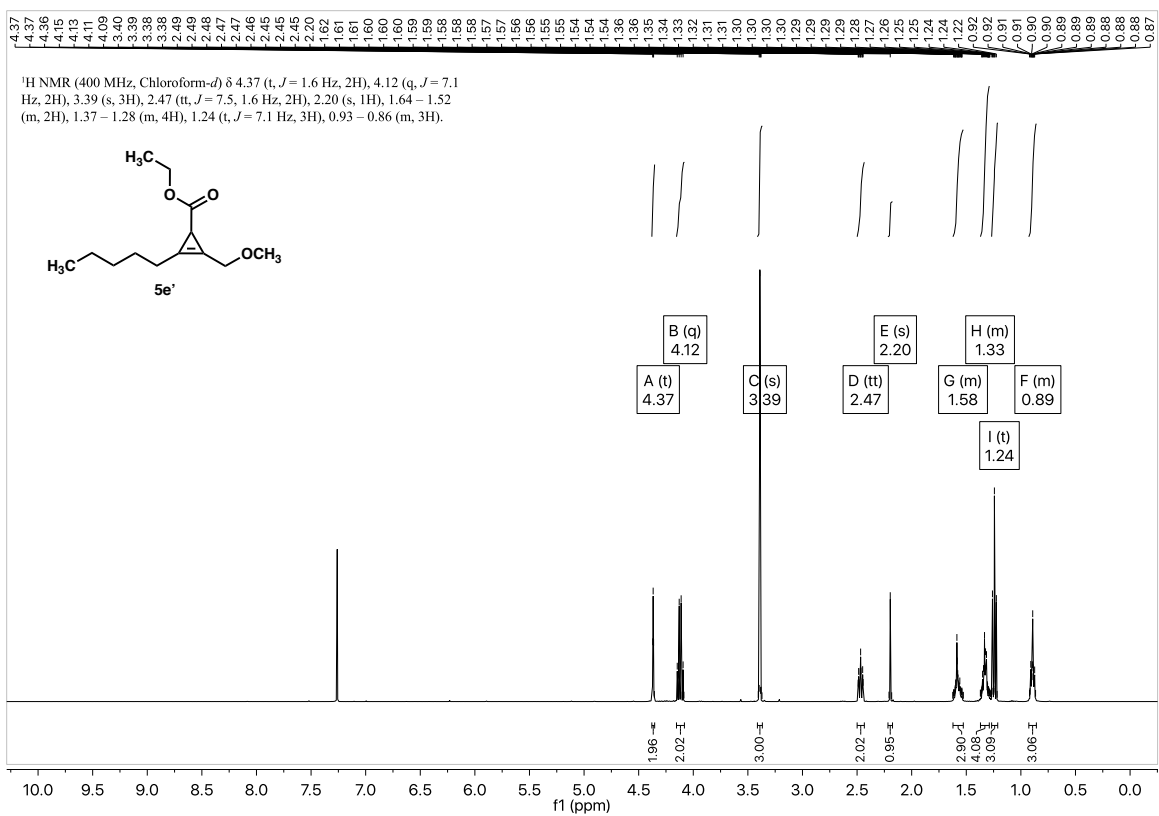


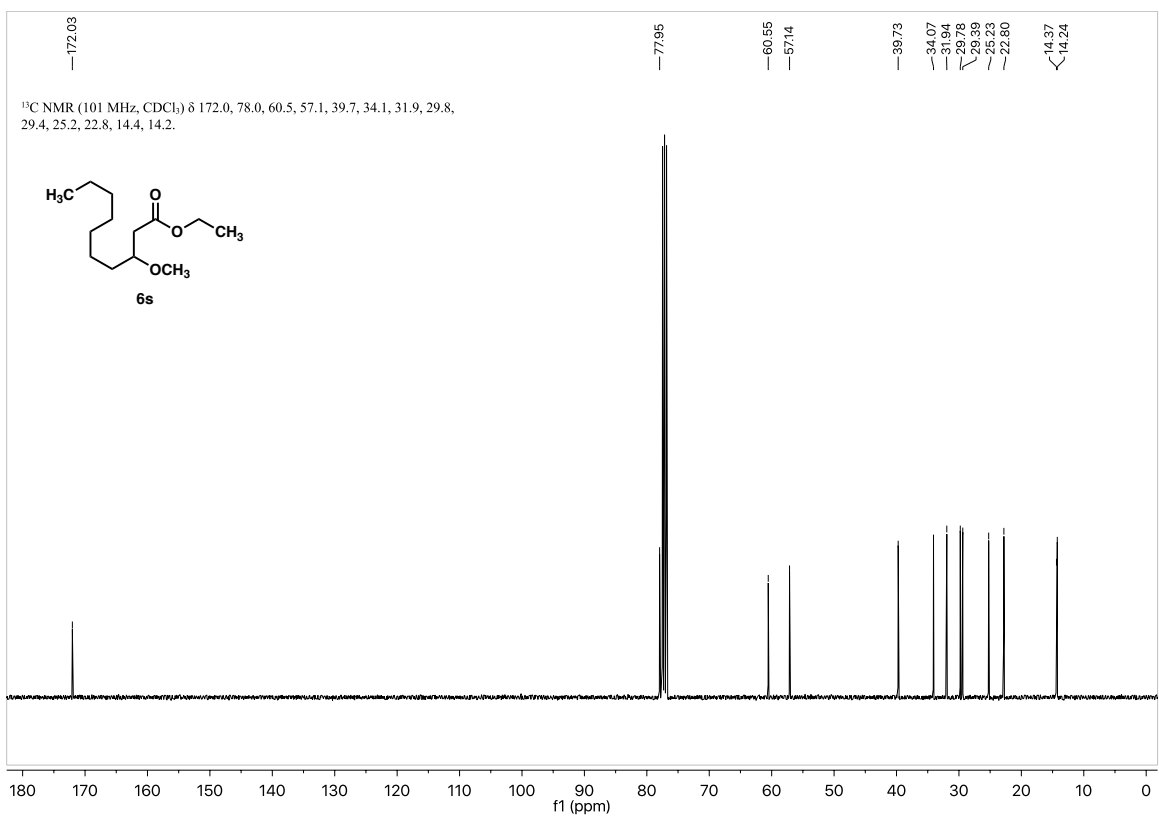
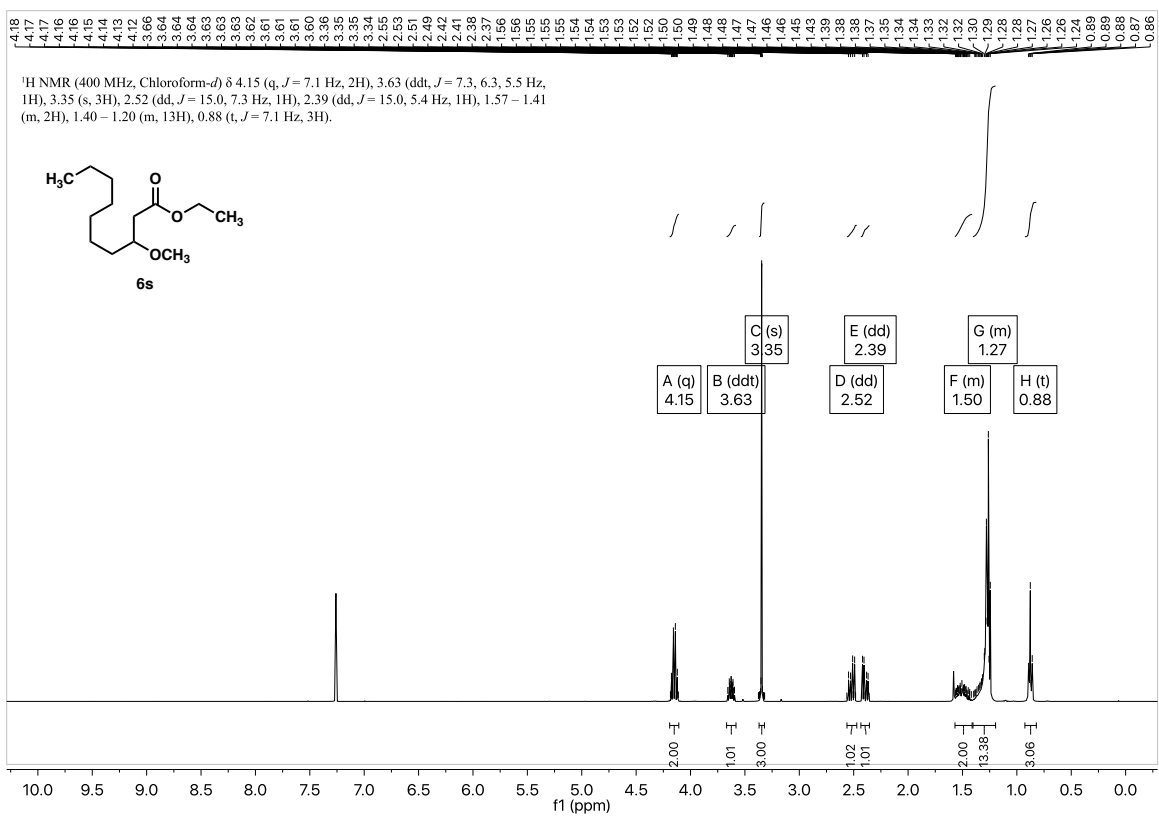


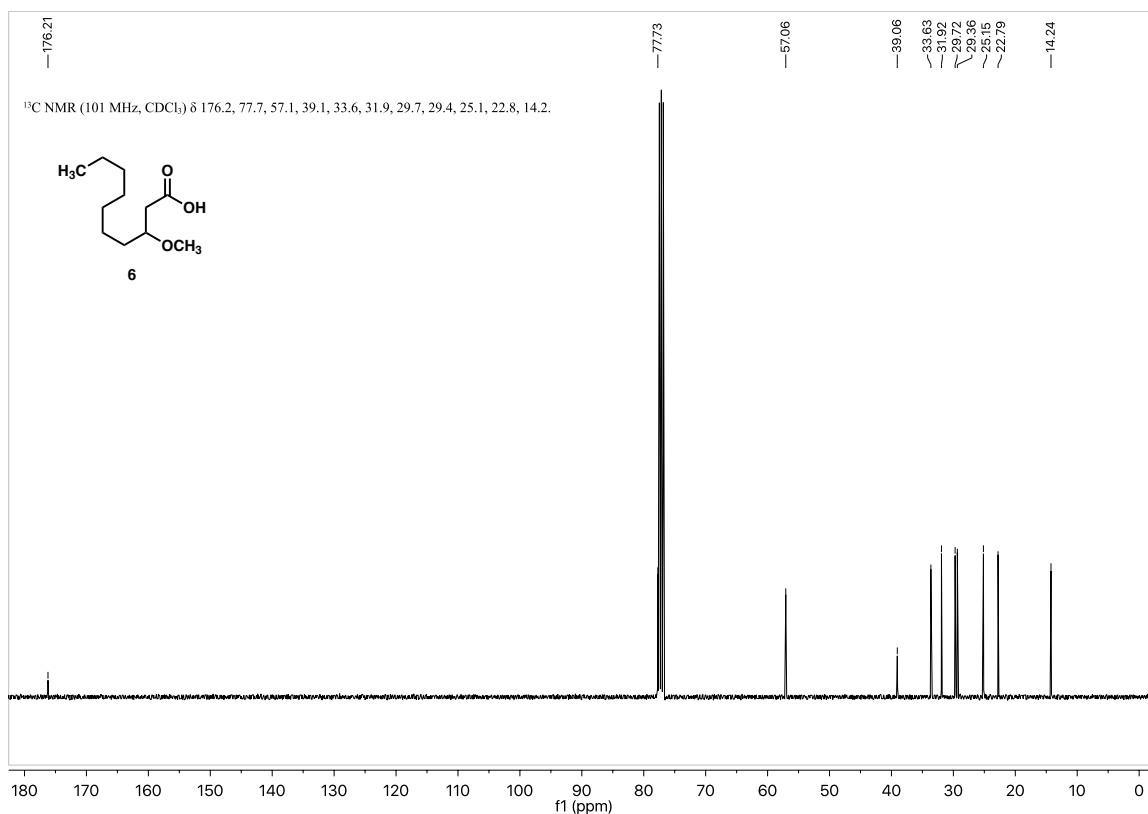
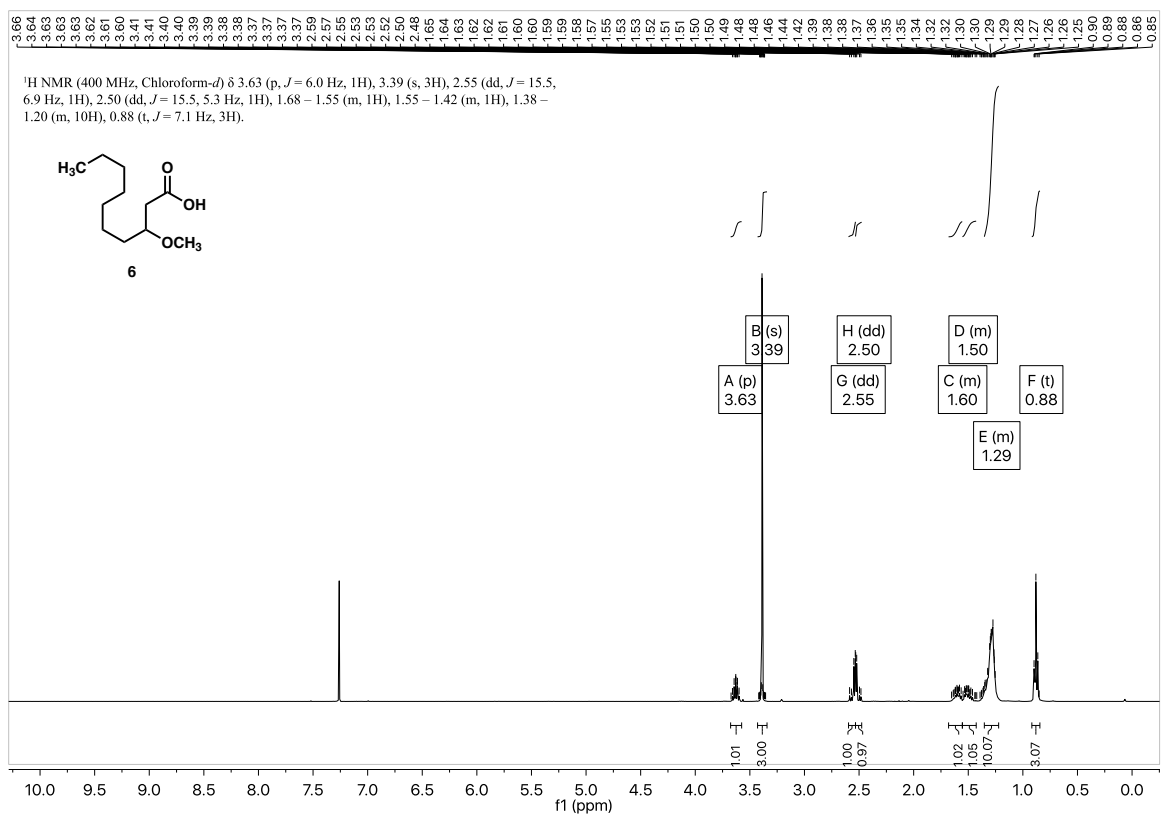


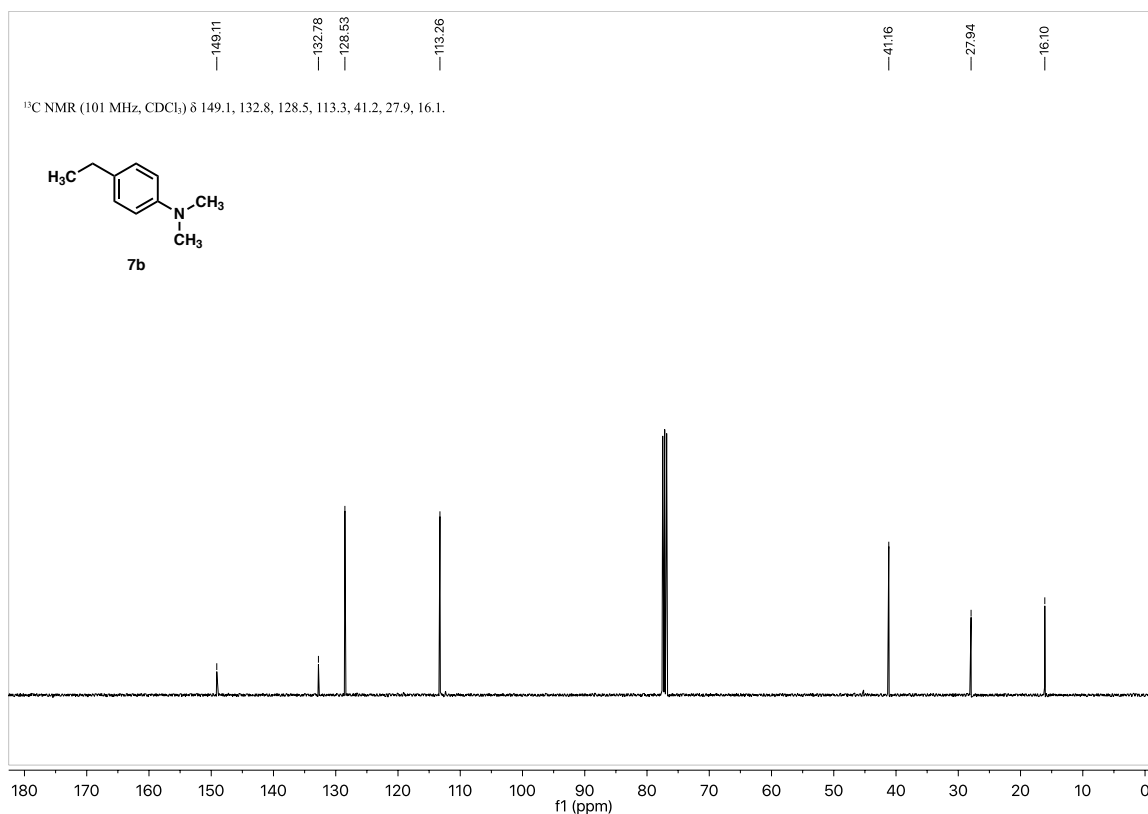
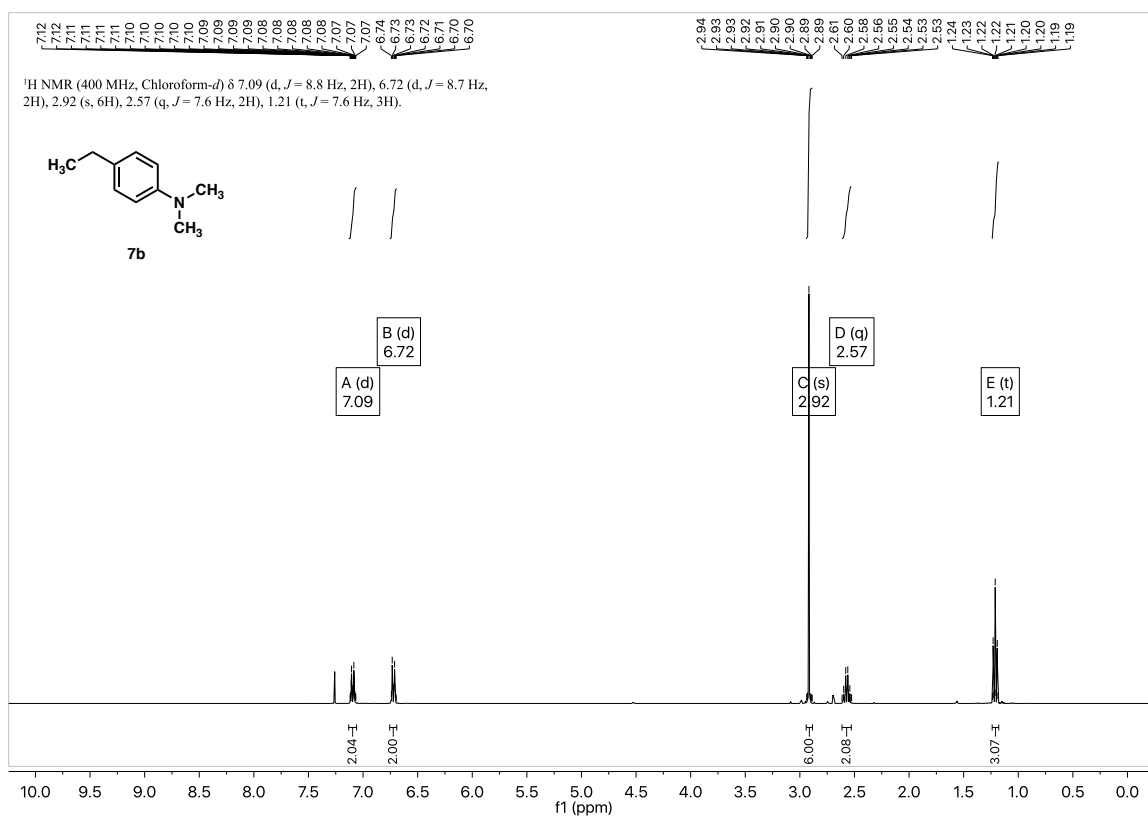


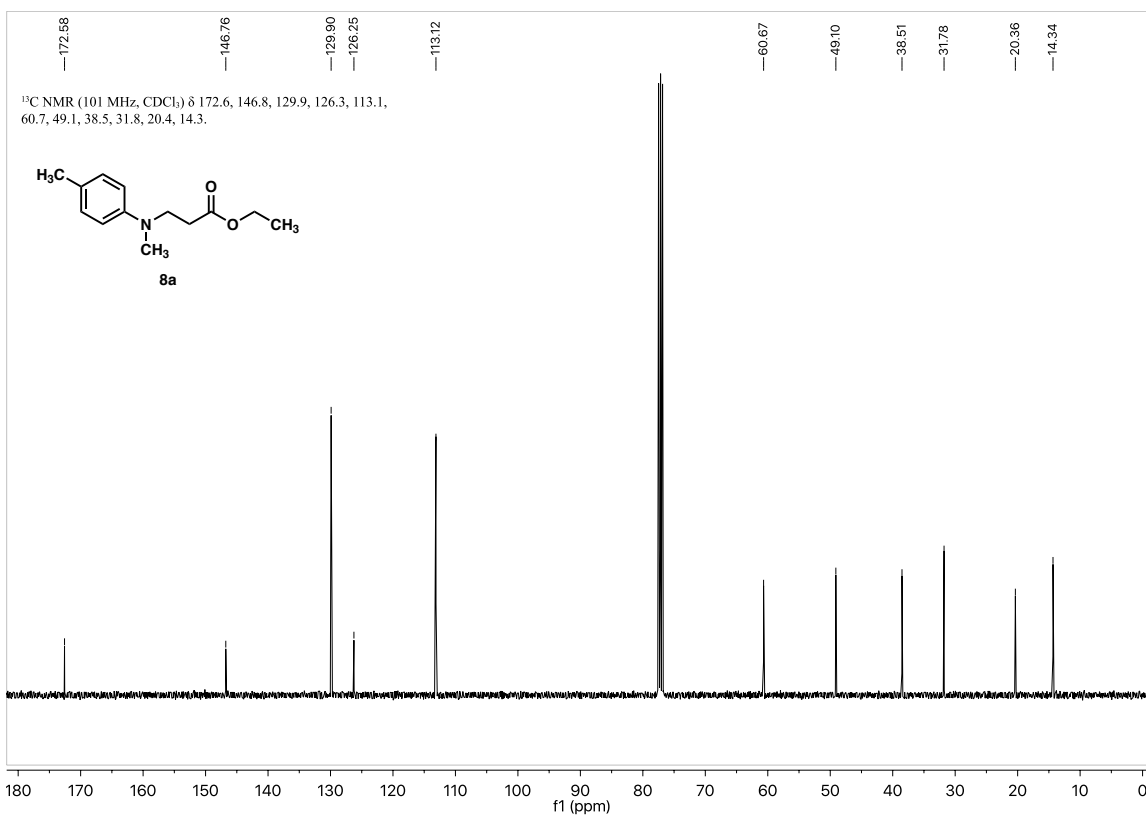
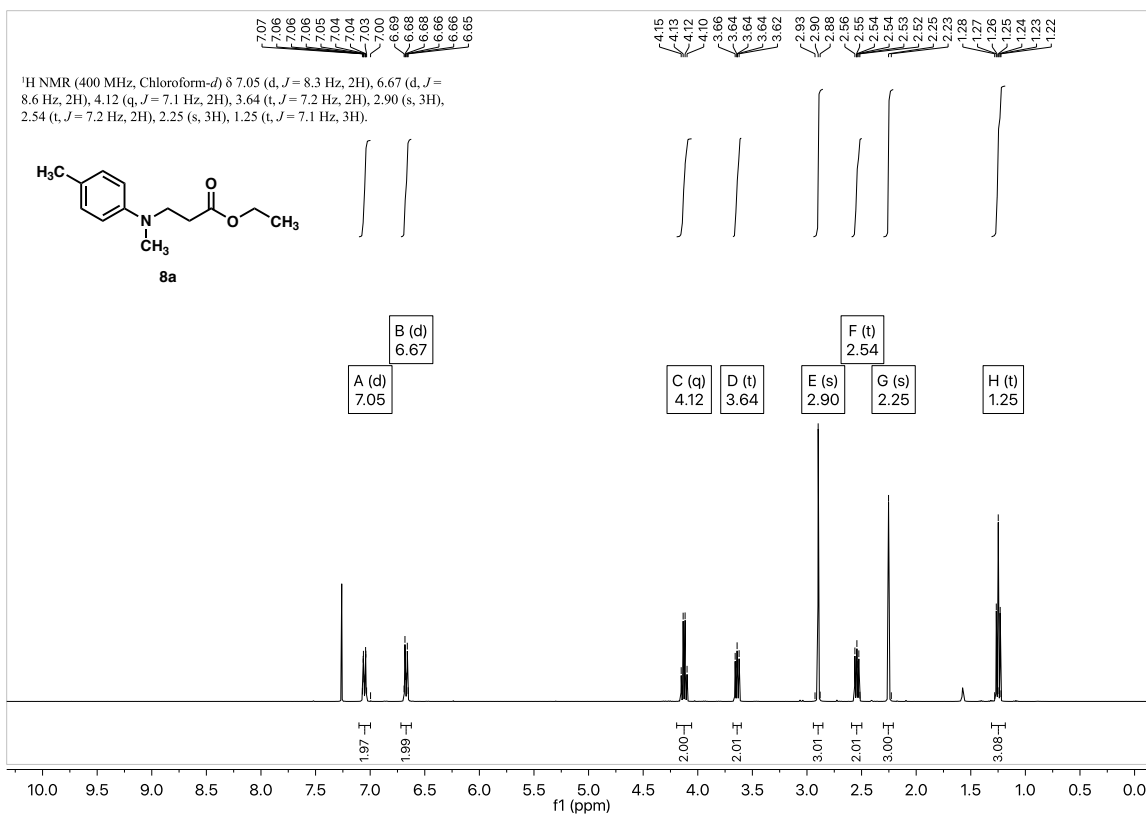


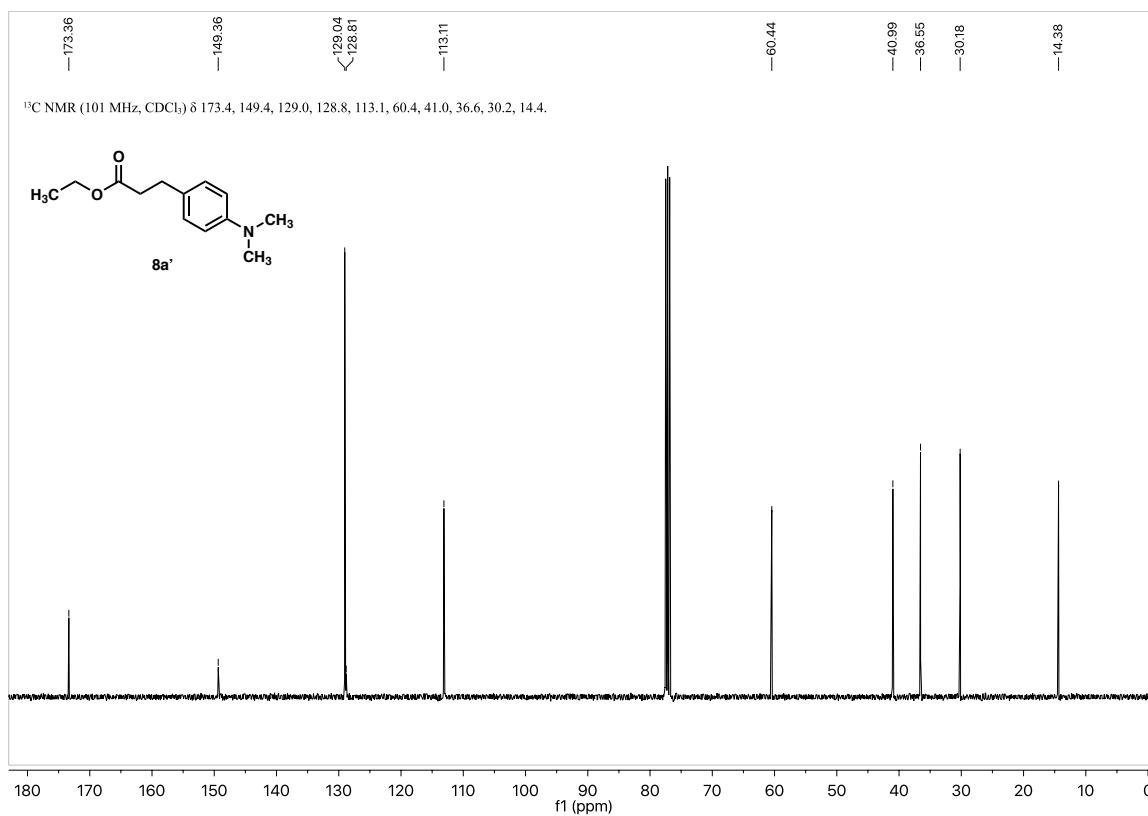
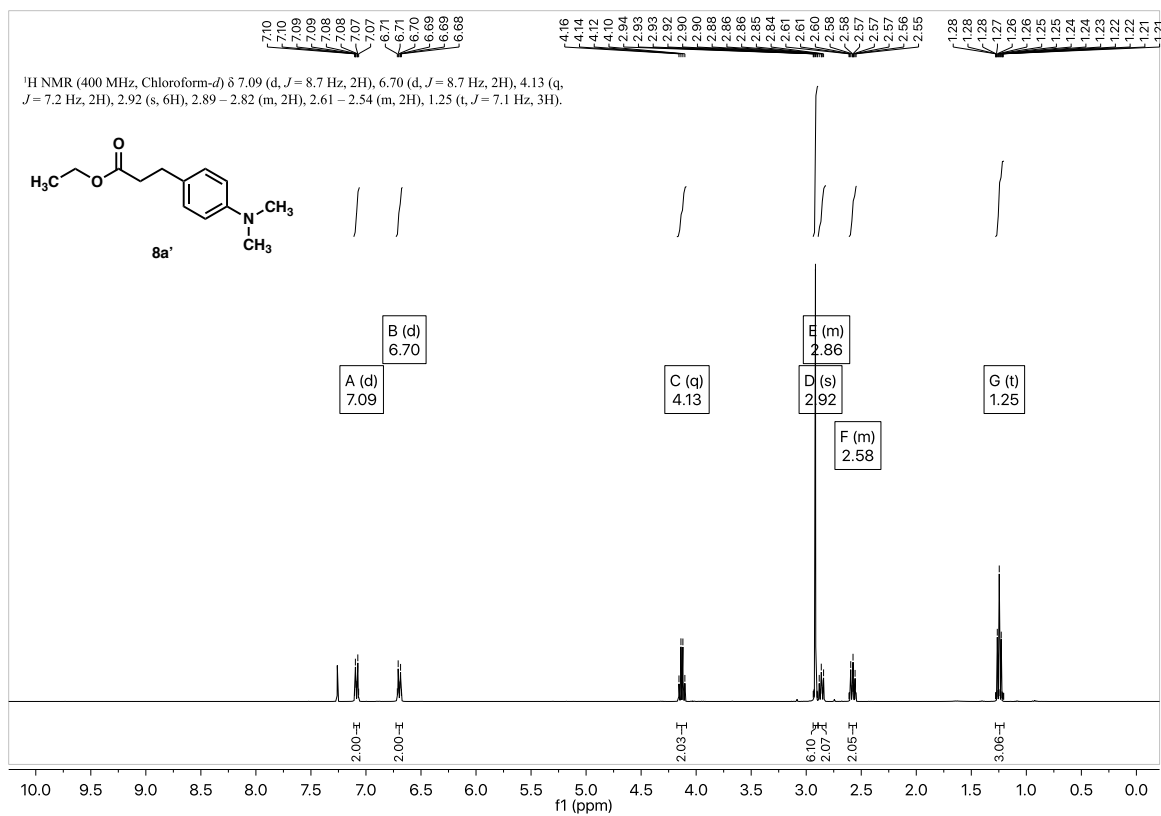


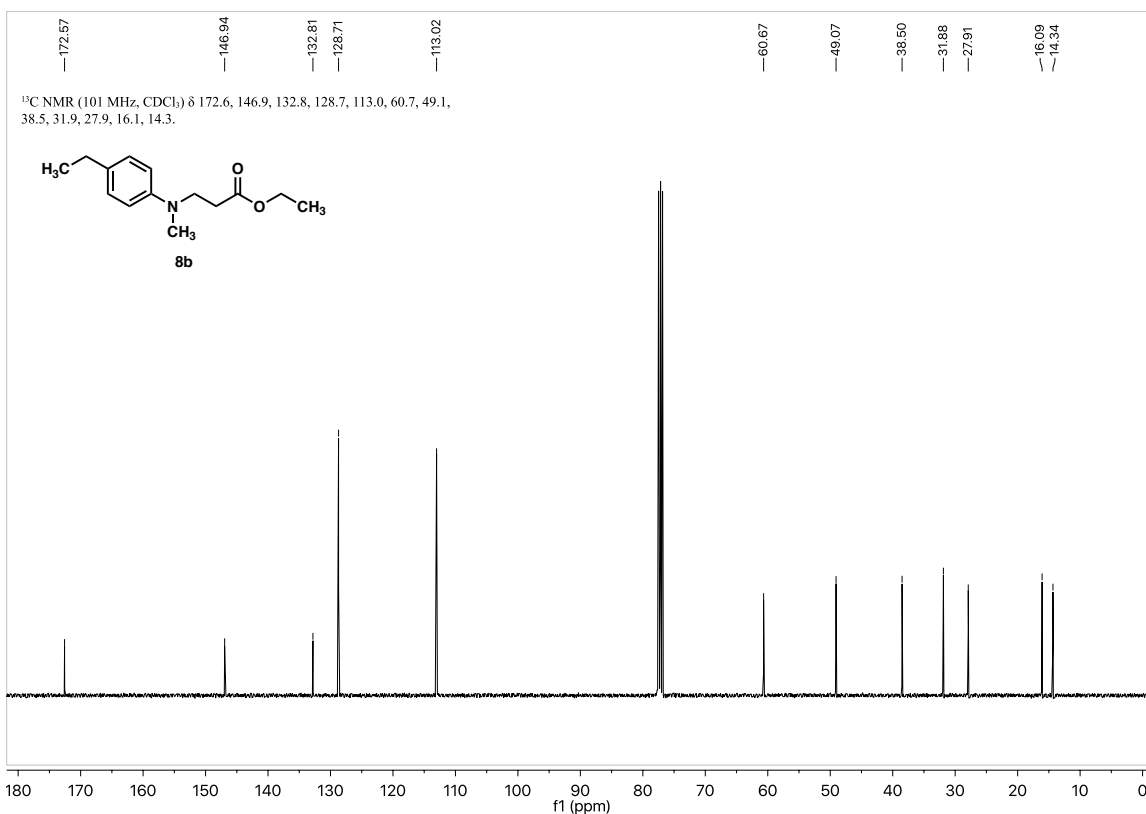
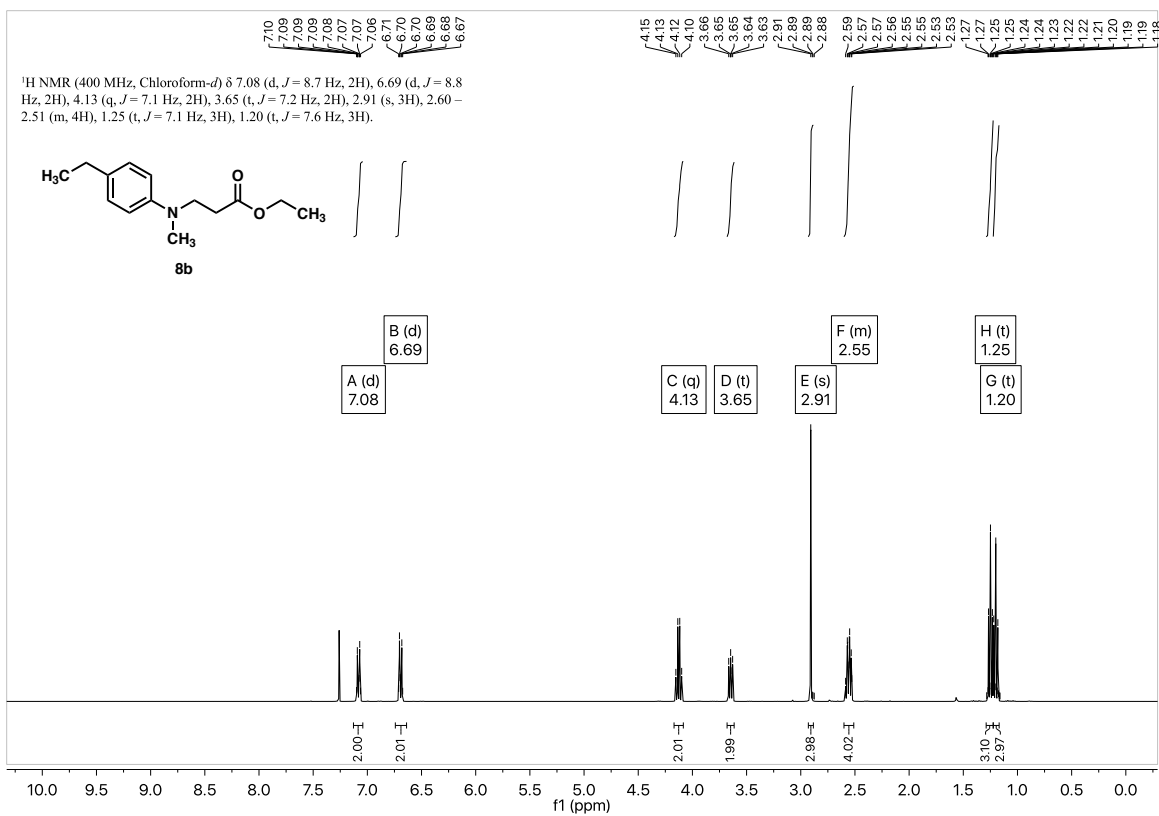


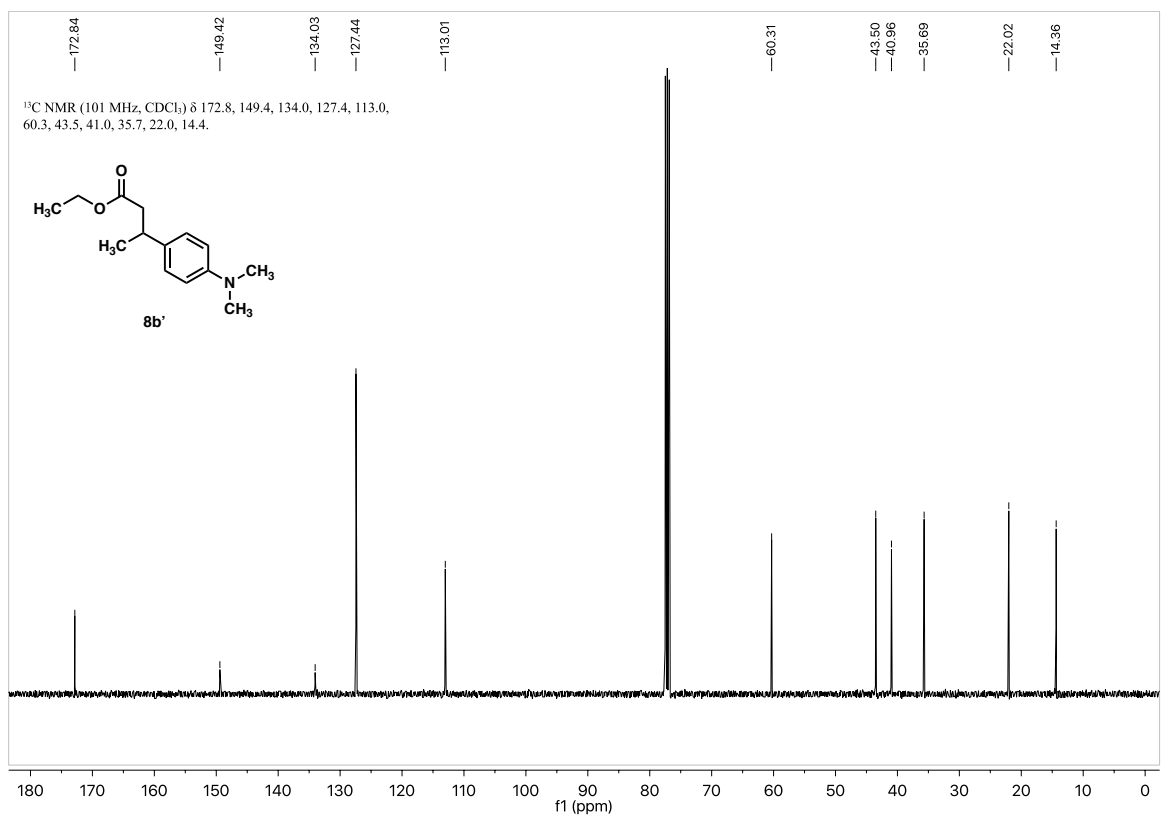
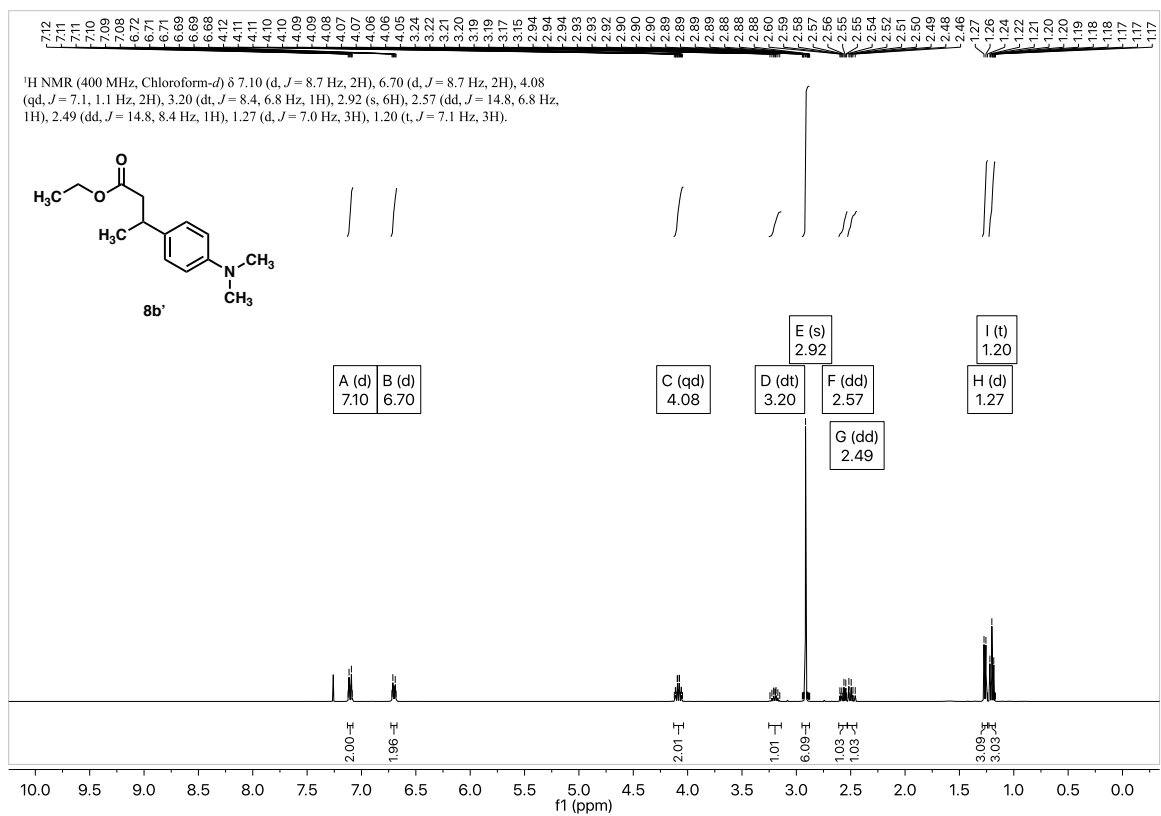


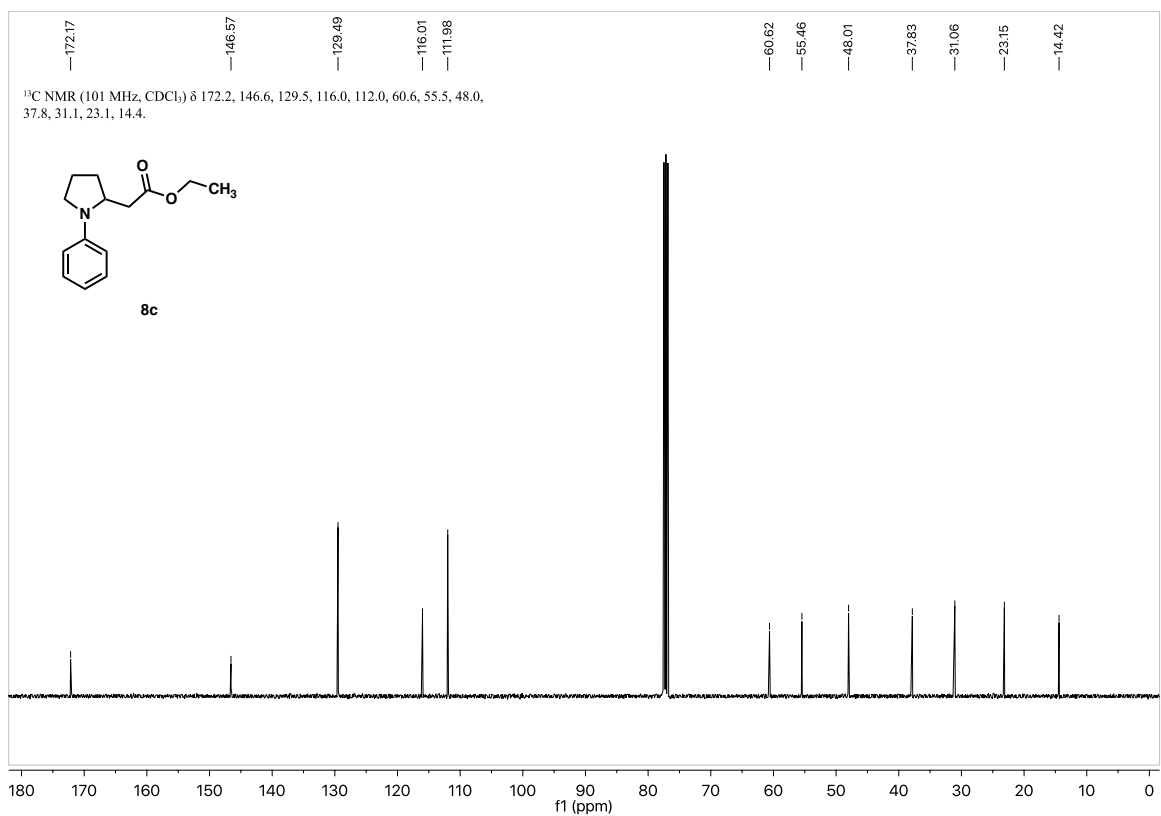
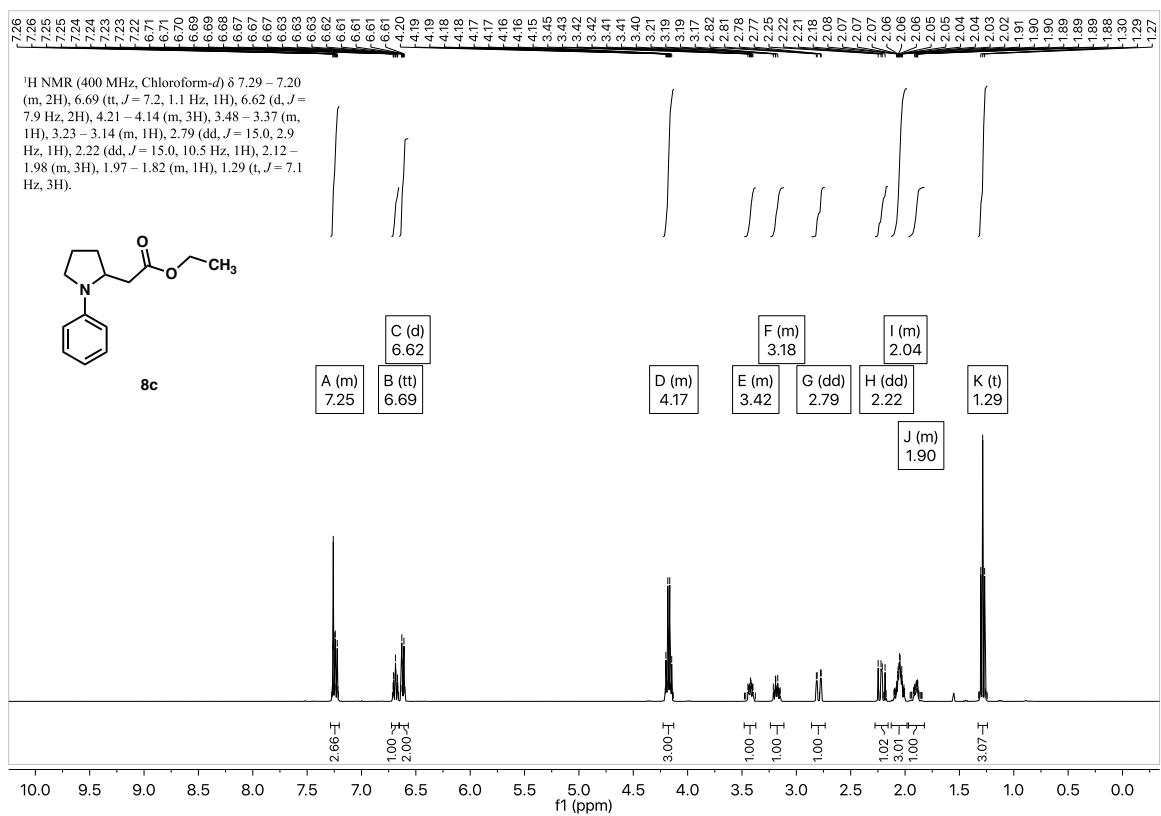


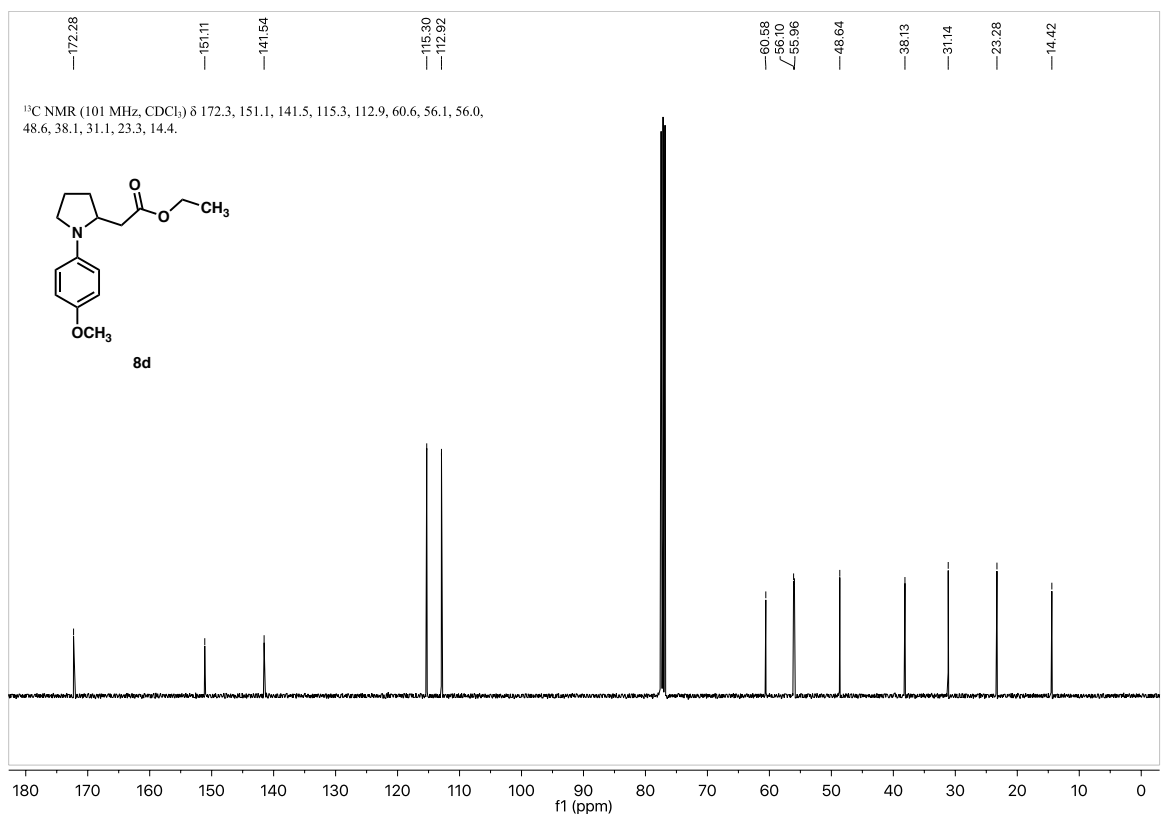
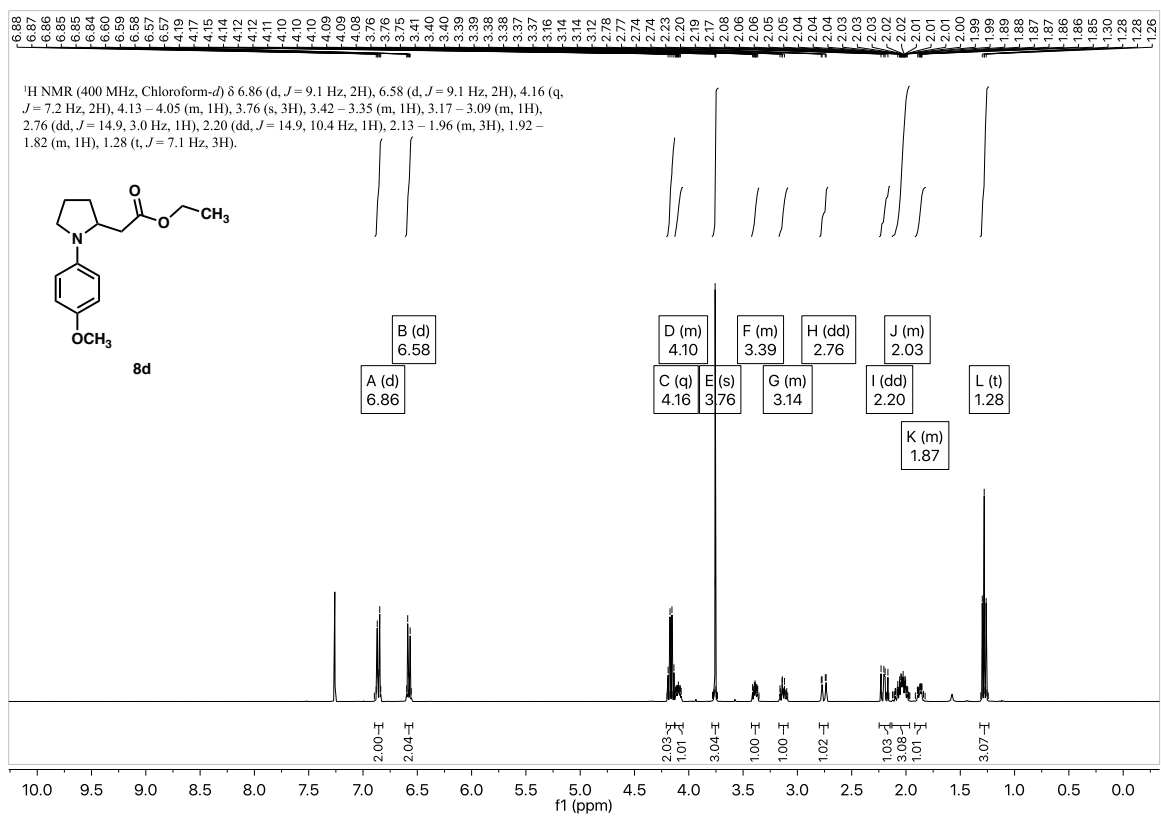


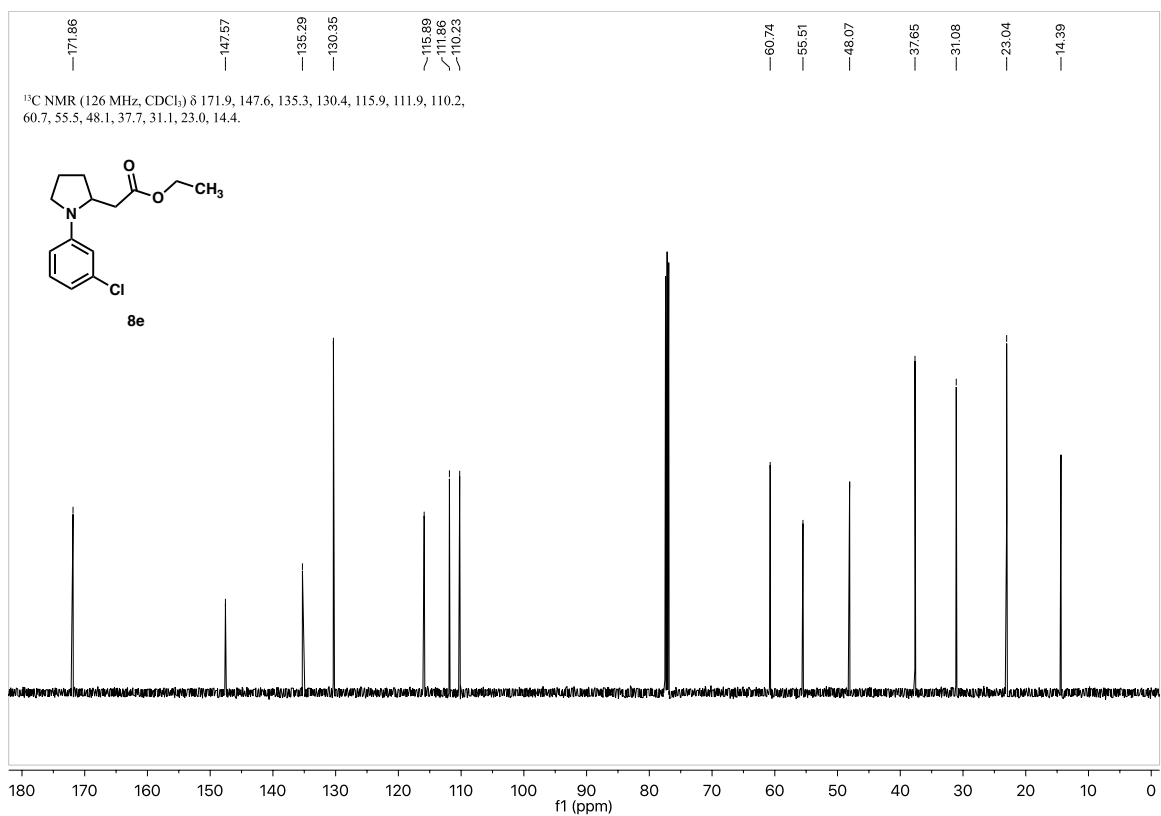
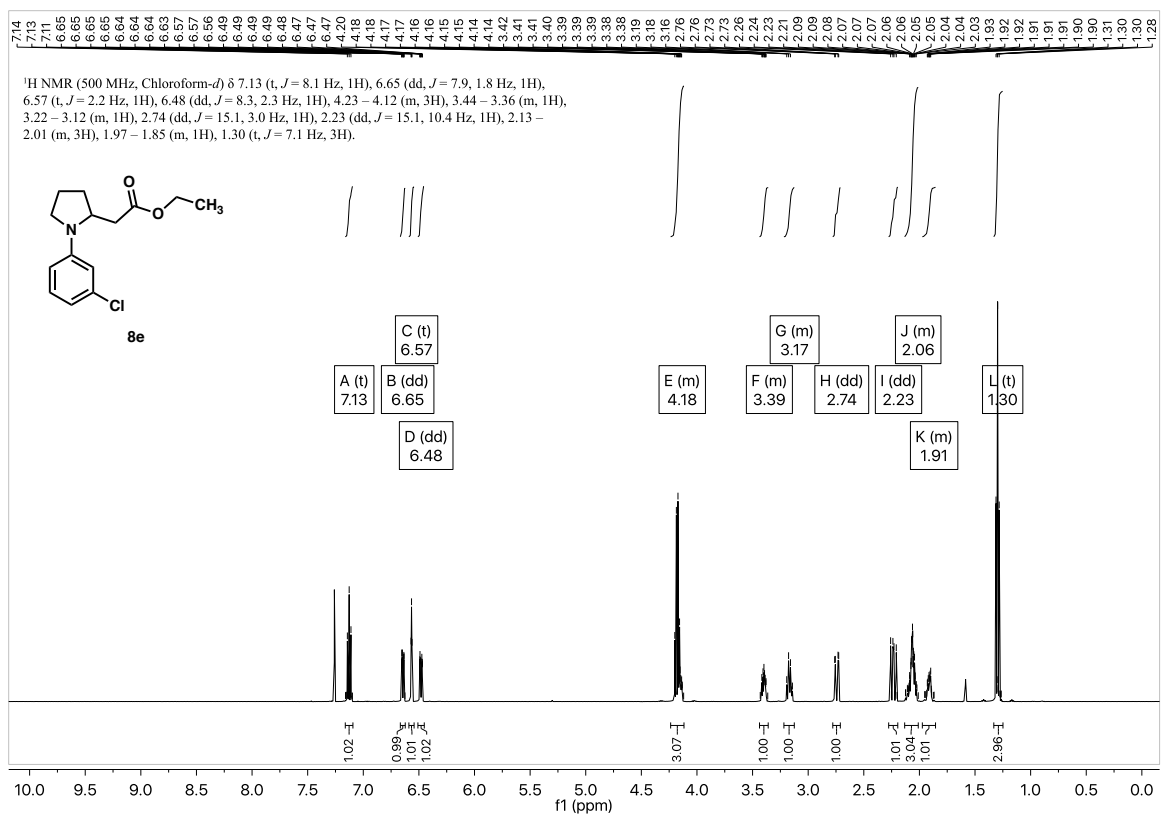


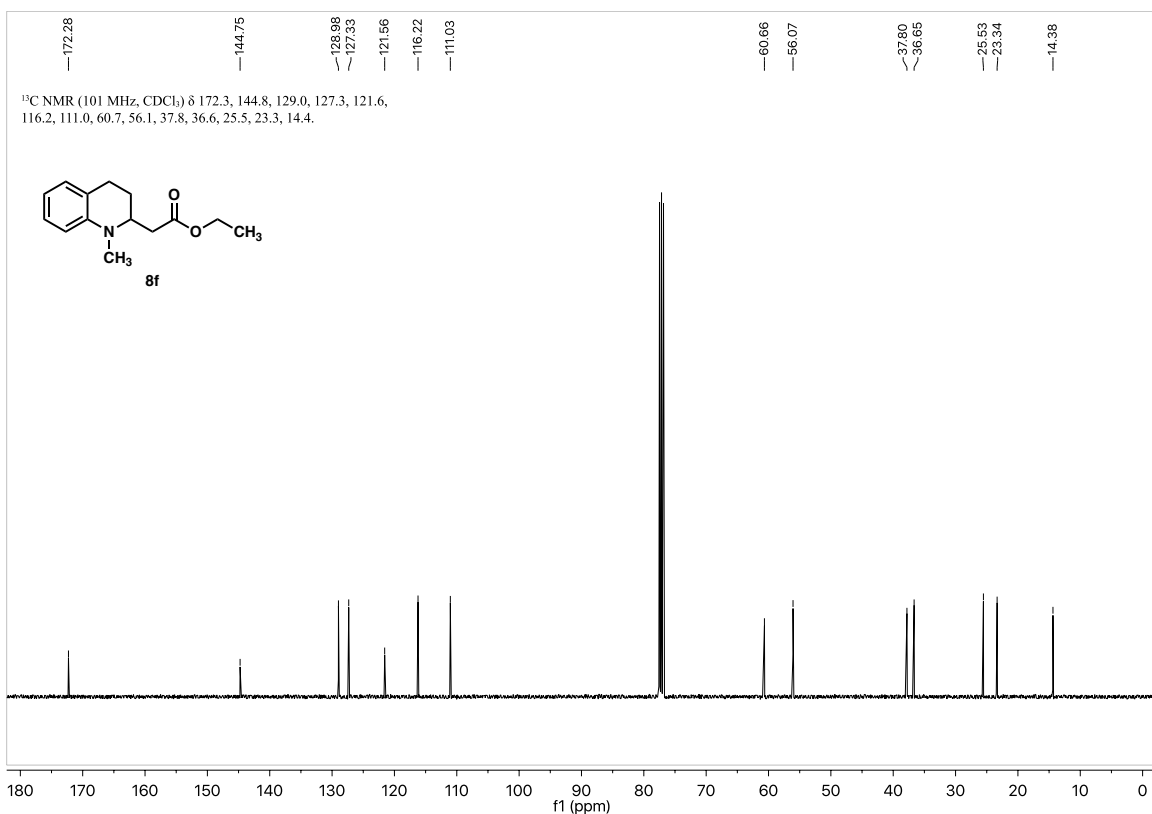
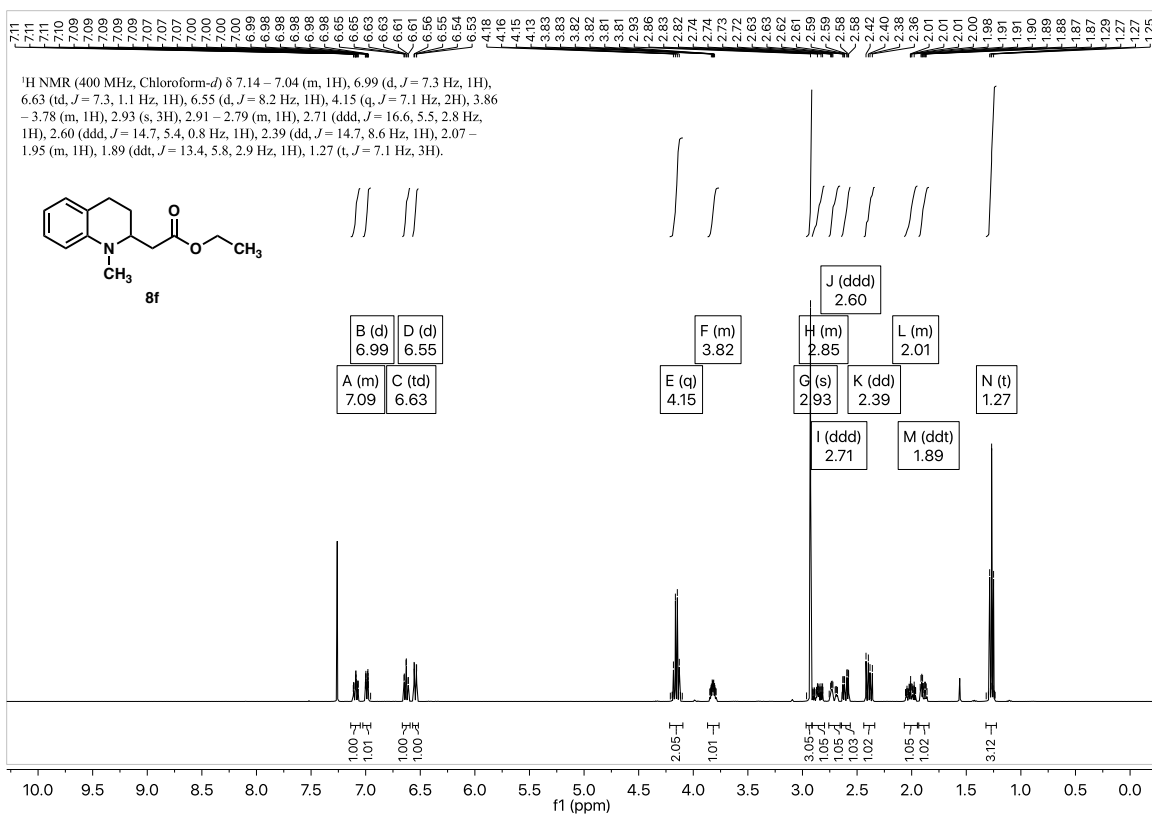


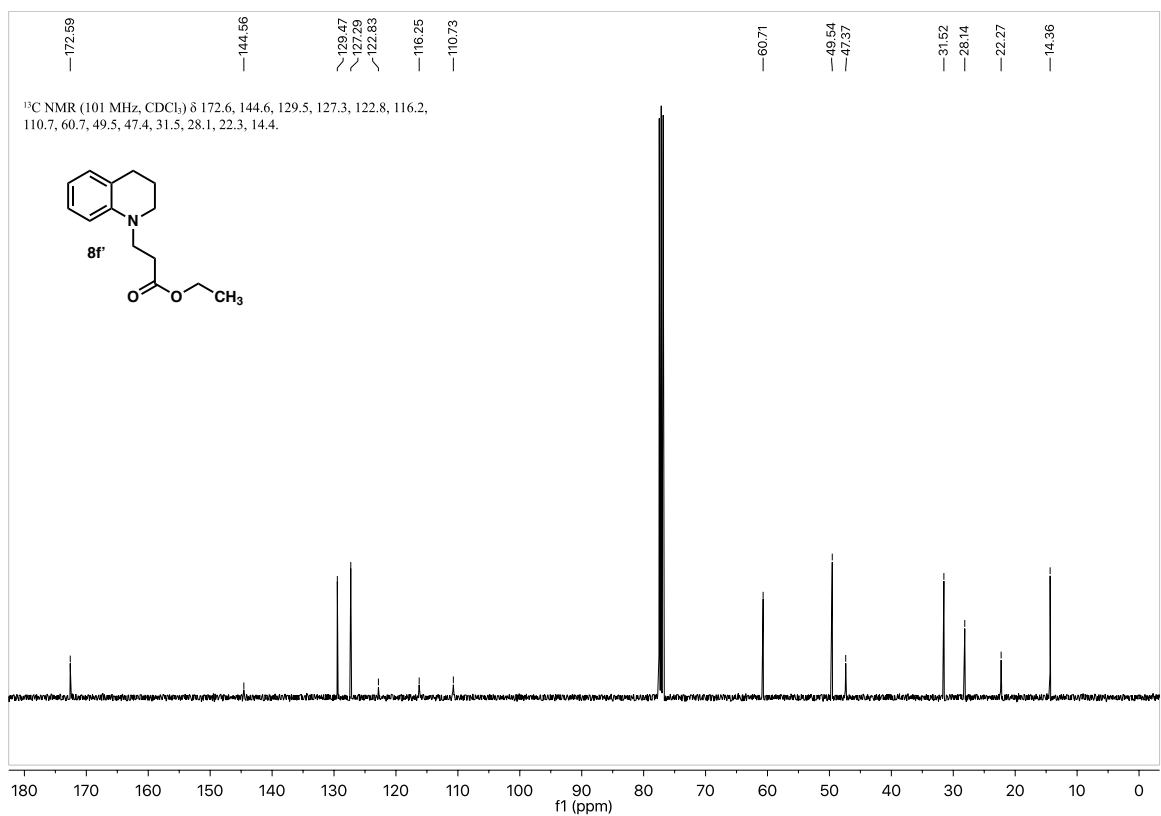
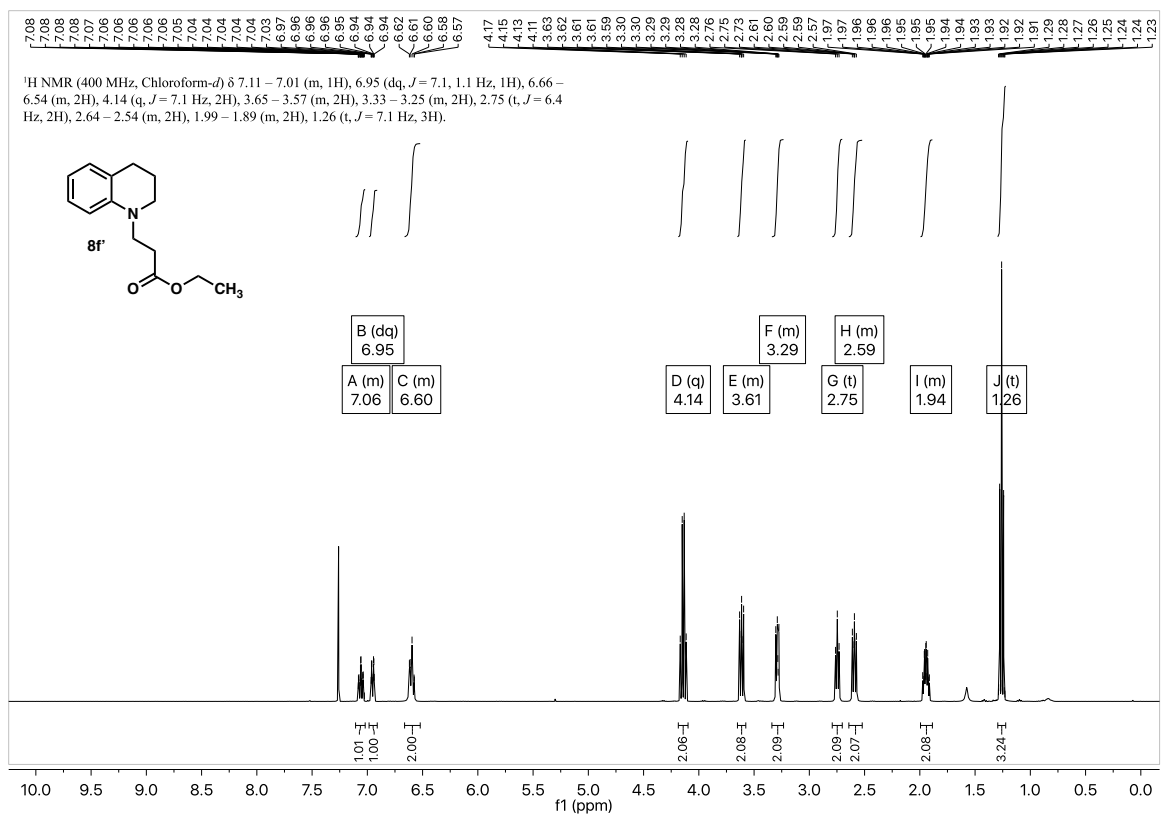


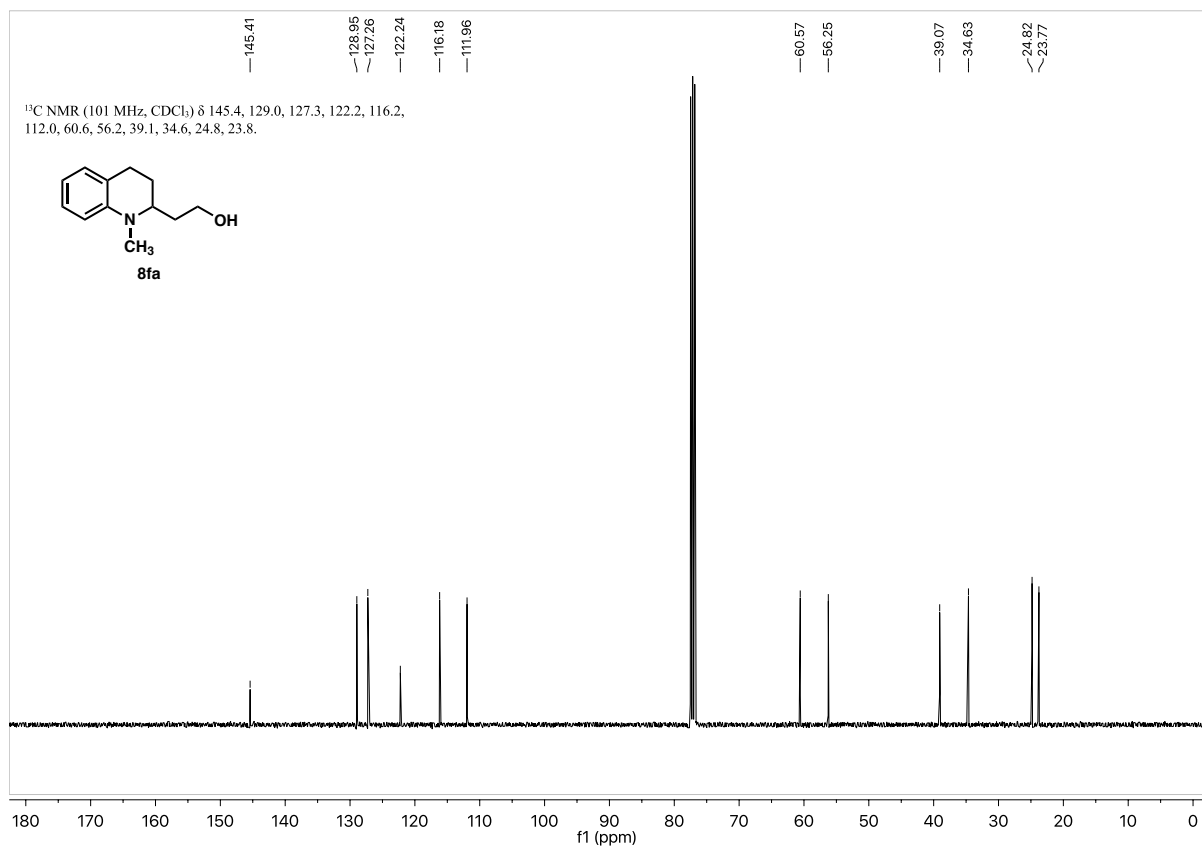
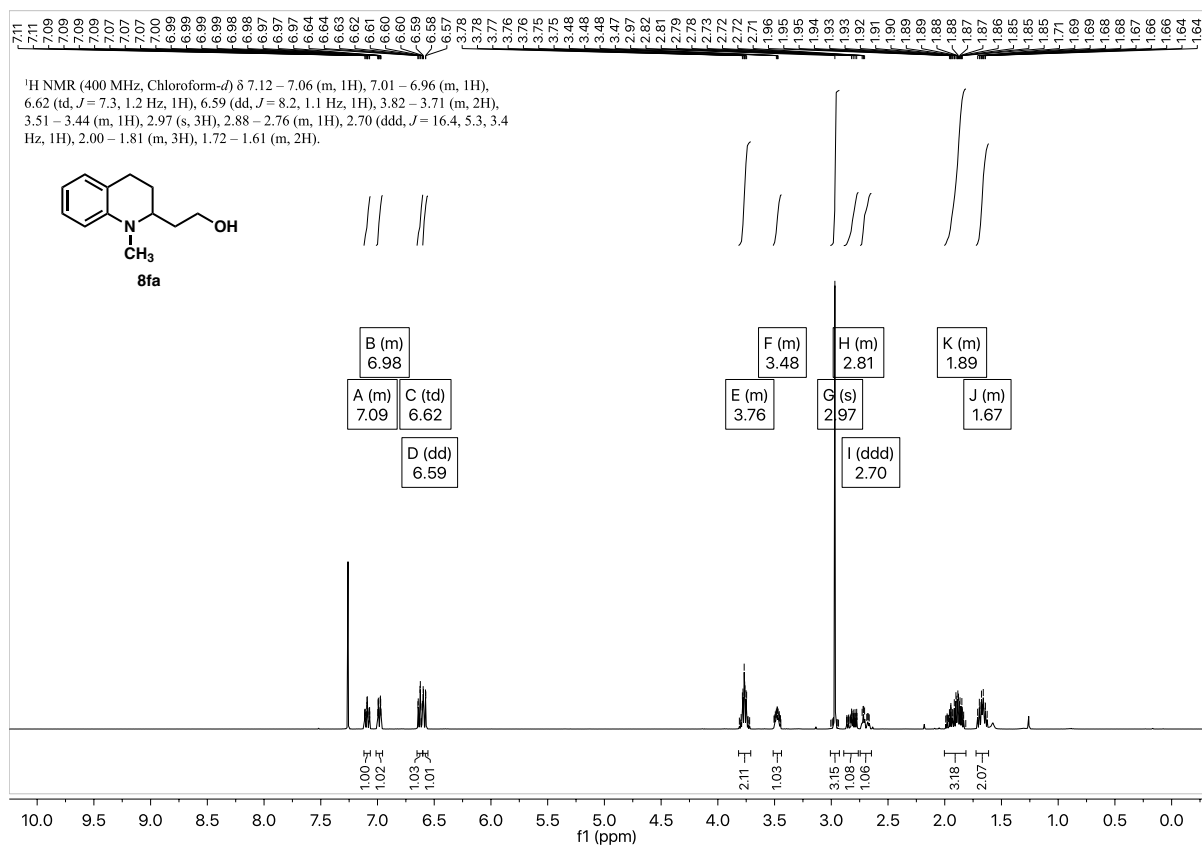


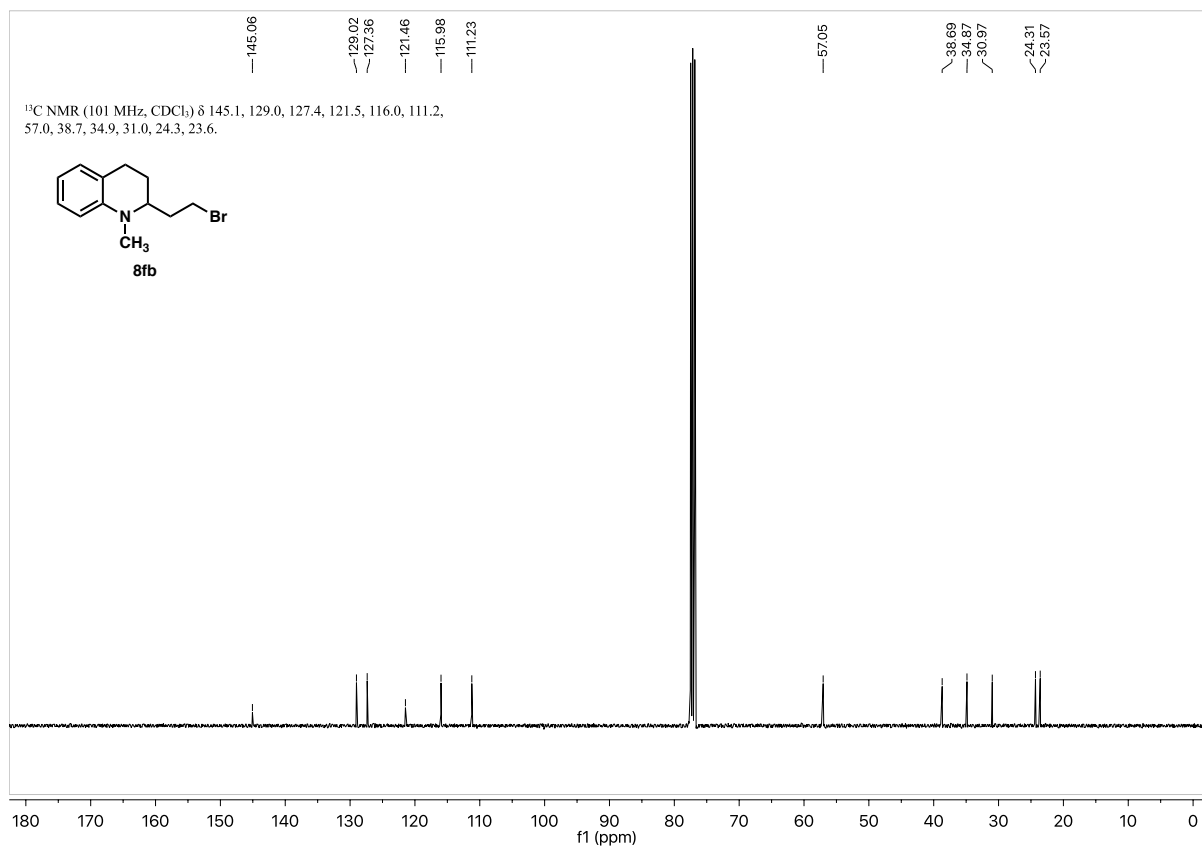
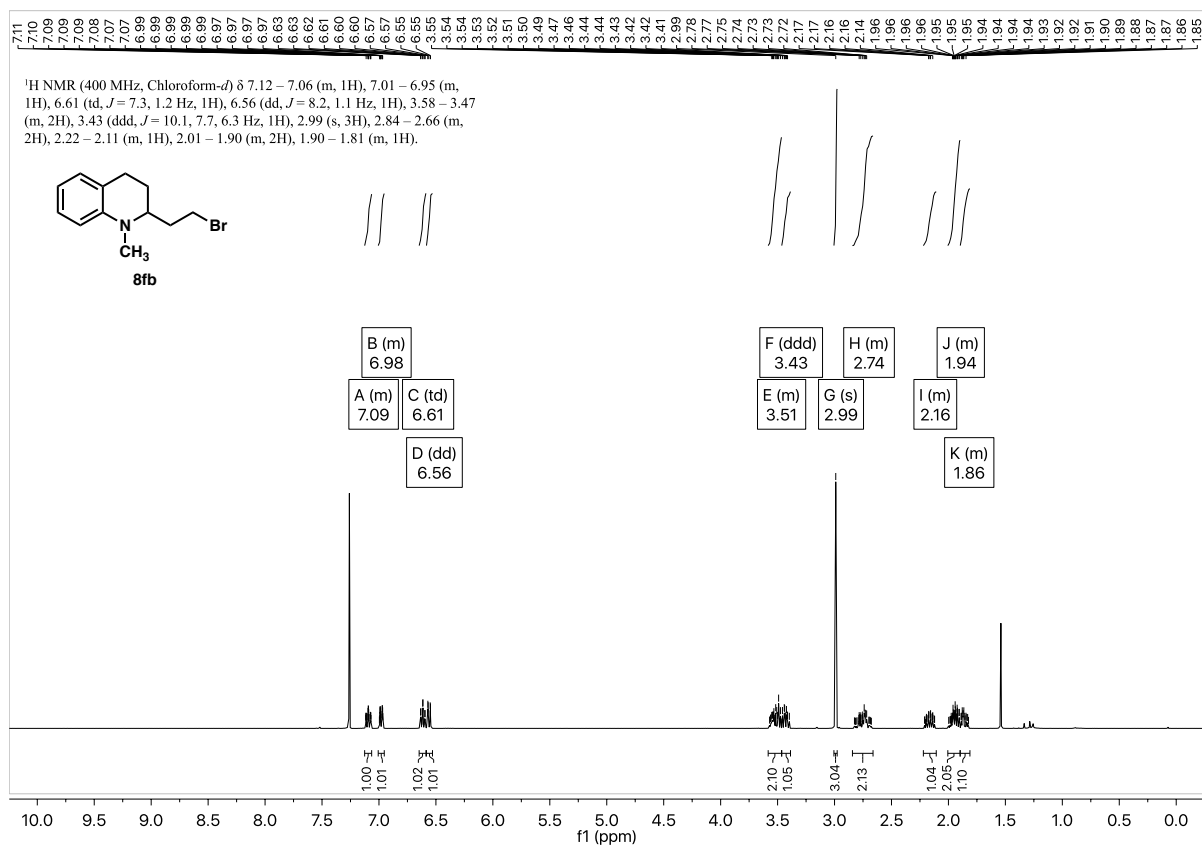


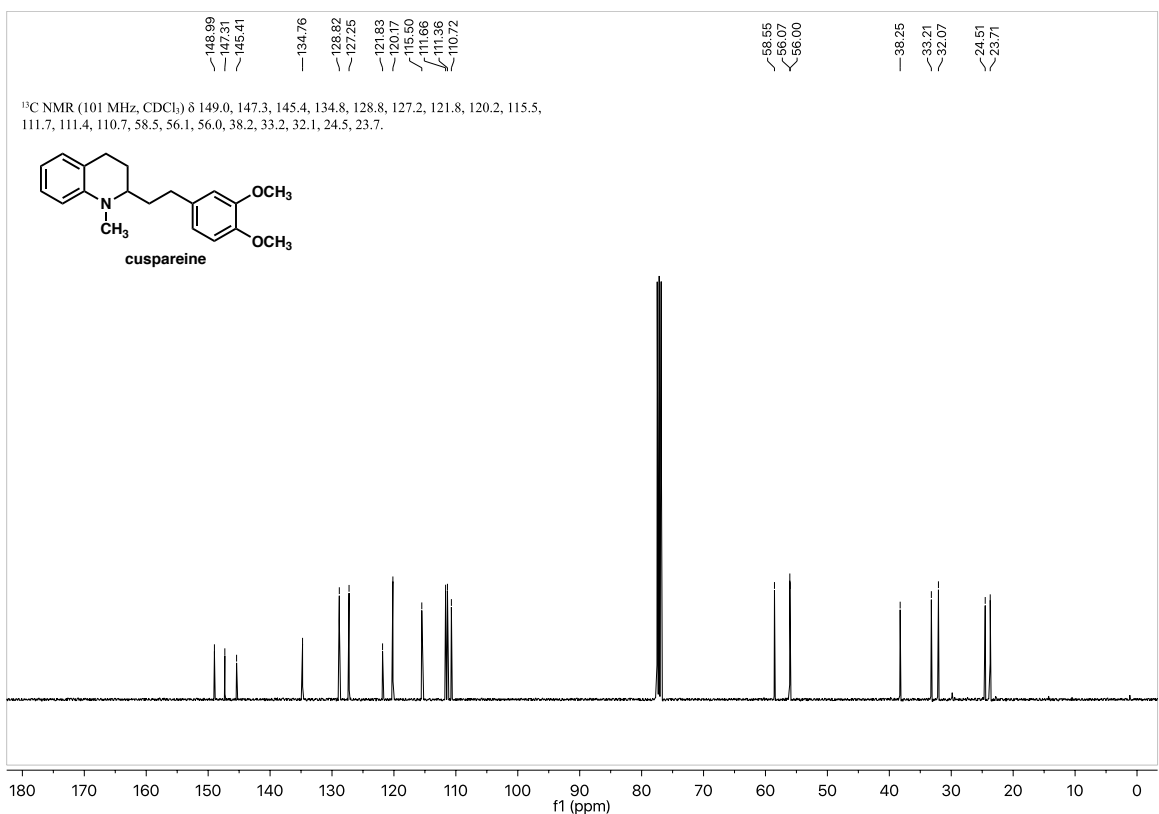
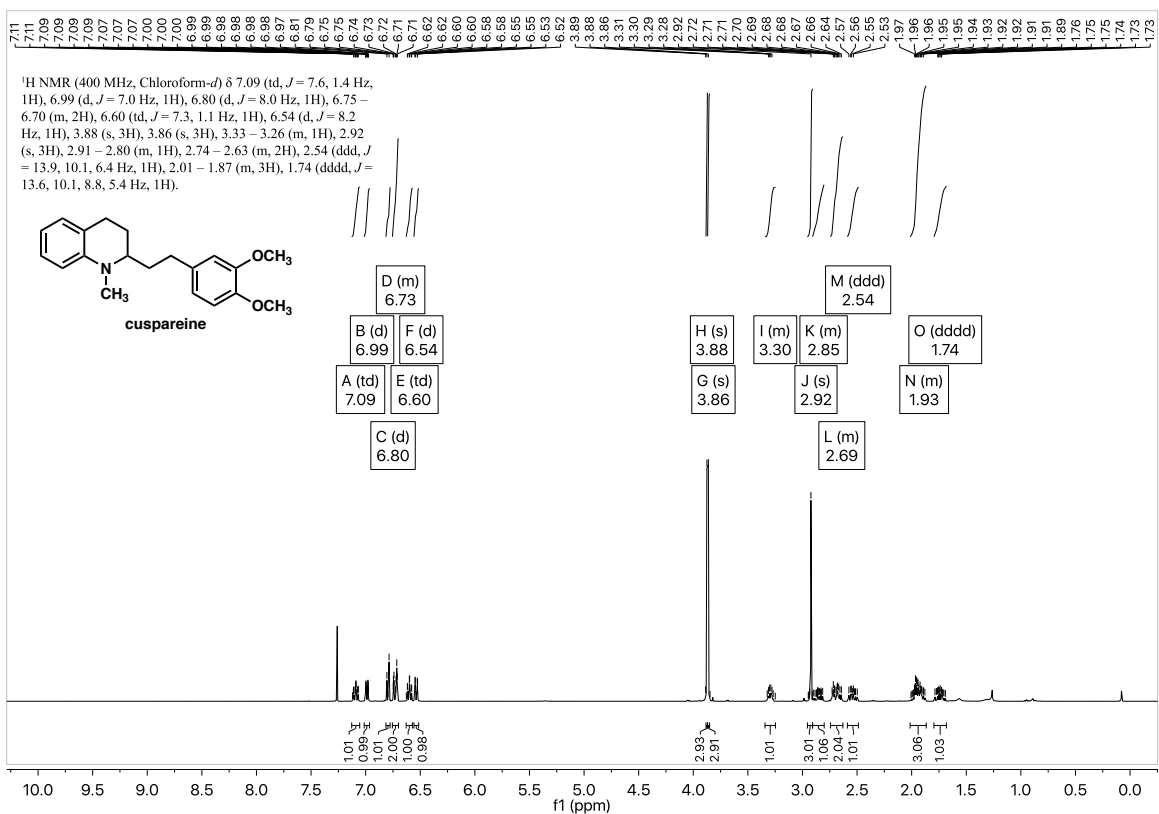


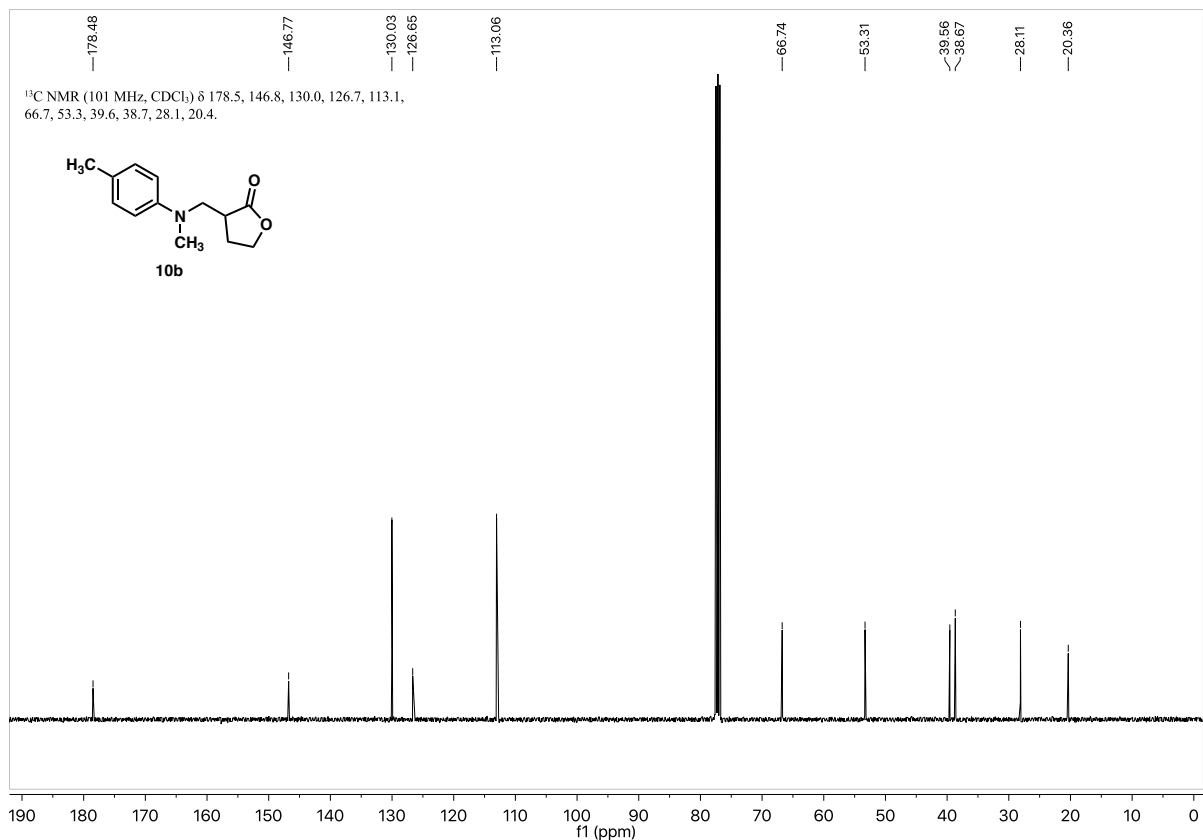
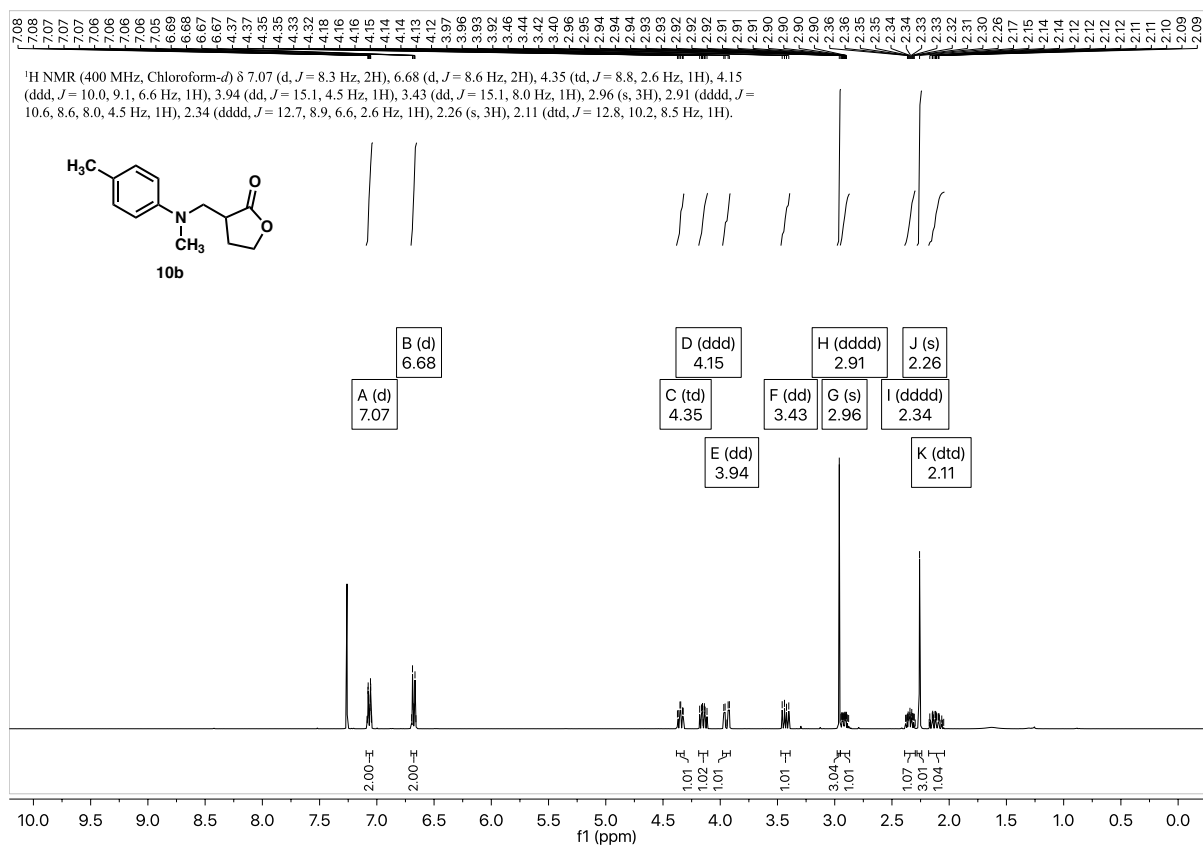


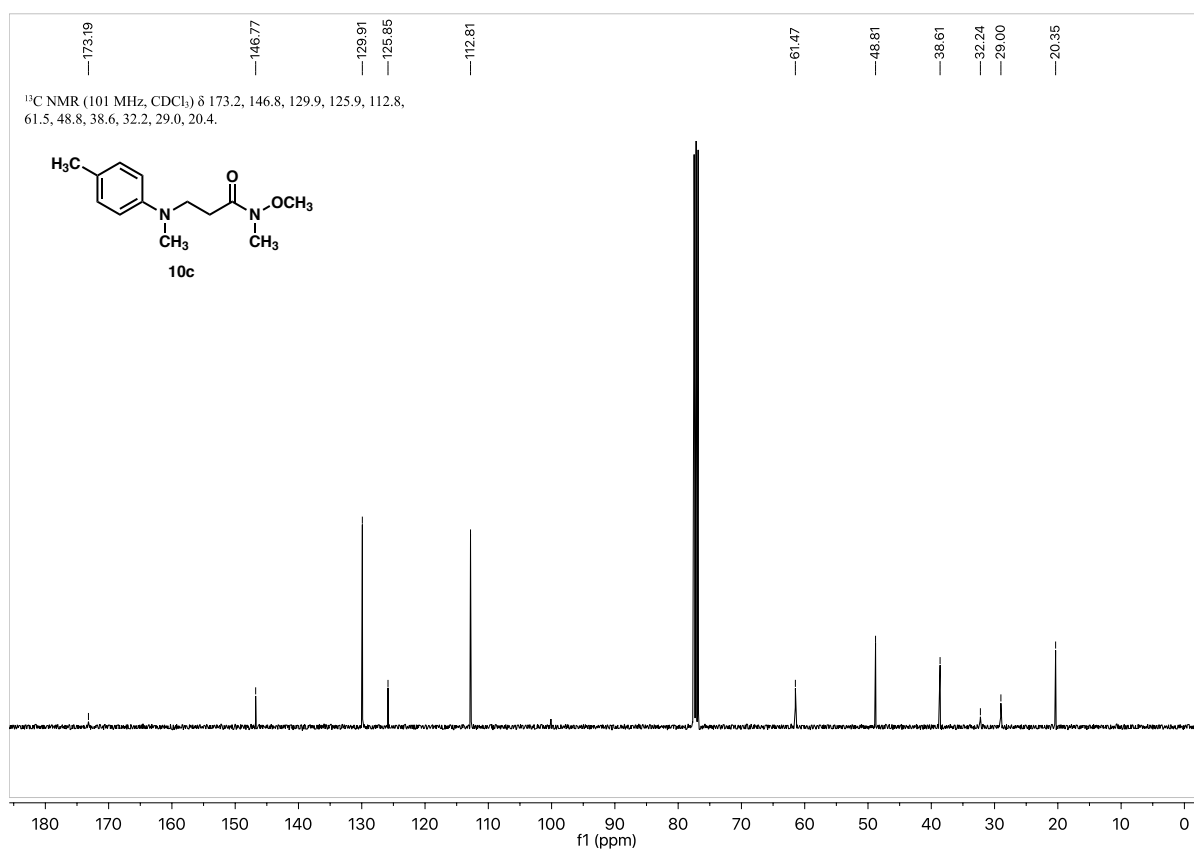
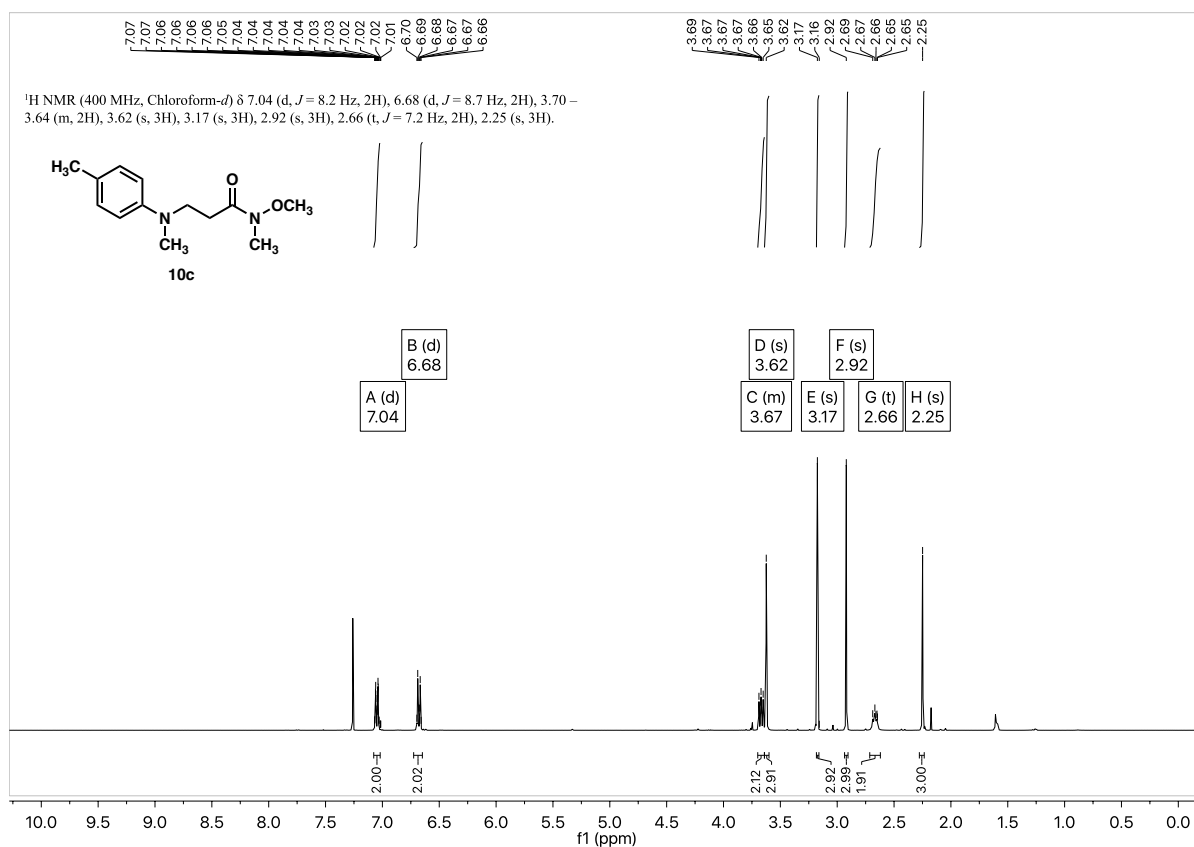


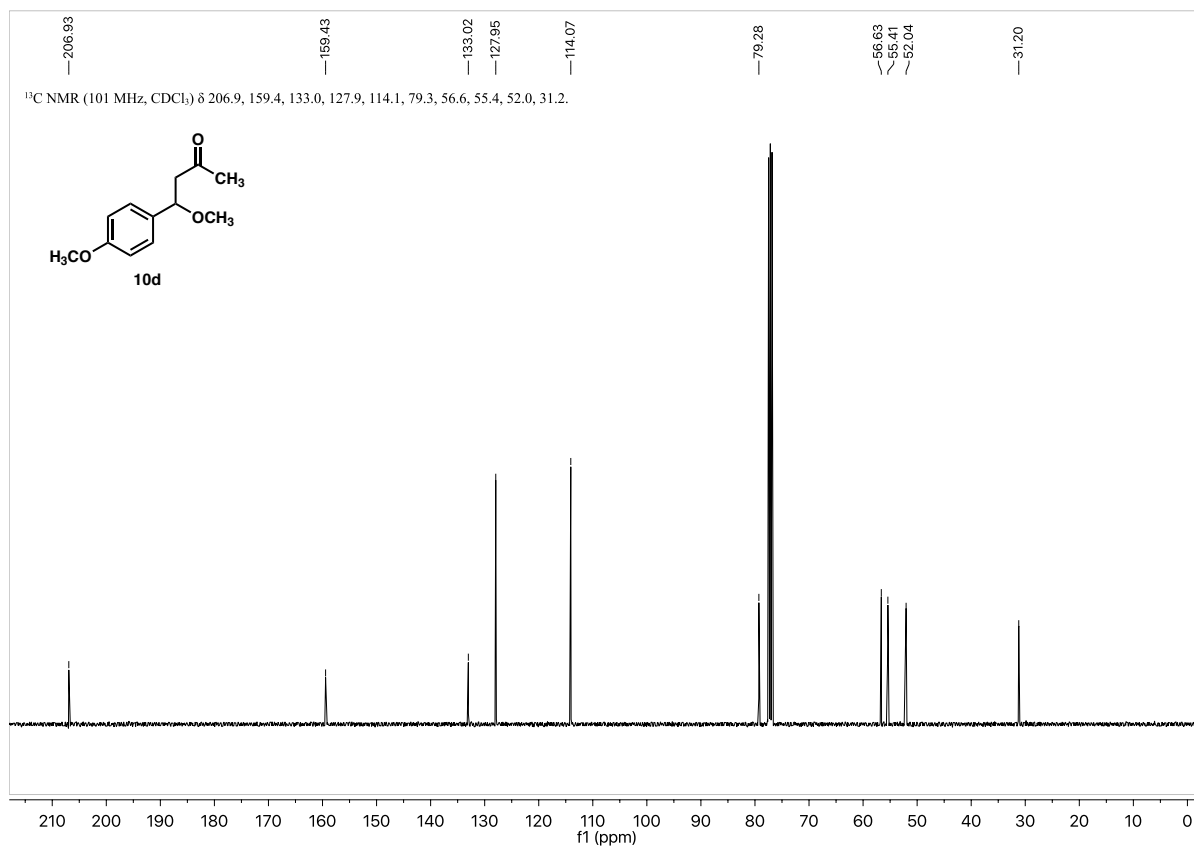
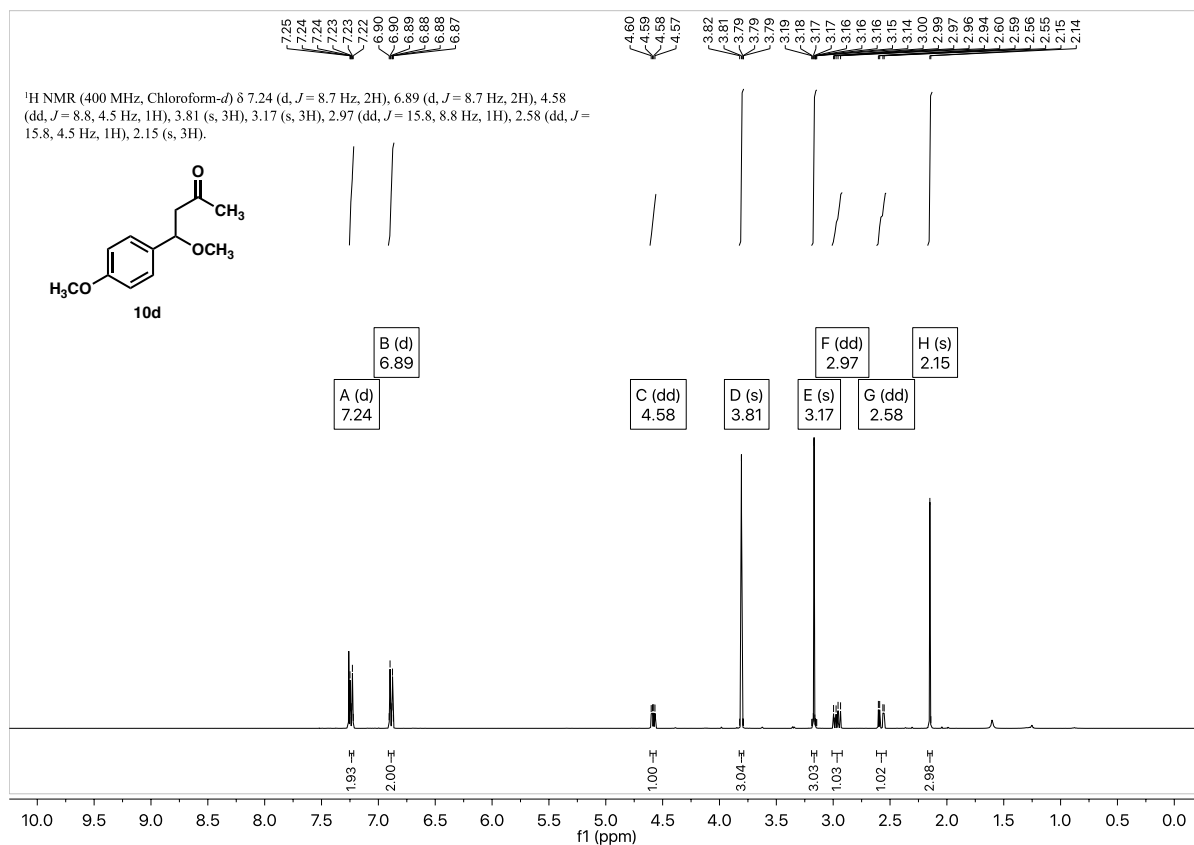












XIII. Supplemental References

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